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A STUDY OF THE METABOLISM OF SINGLE THERAPEUTIC DOSES (2.0 MG.) OF ESTRONE IN THE PREOVULATORY AND POSTOVULATORY PHASES OF THE MENSTRUAL CYCLE IN HEALTHY WOMEN*

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ZONDEK'S demonstration in 1934 (1) of the tremendous capacity of the rat to inactivate the natural estrogens has focused attention on the intermediary metabolism of these hormones. Treatment and recovery experiments in man (2–5) have revealed that, regardless of sex, only from 0 to 20 per cent of the administered hormone can be recovered from the urine in recognizable forms and that α-estradiol$\rightleftarrows$estrone$\rightarrow$estriol conversion appears to characterize one phase of this catabolic process. Although interest recently has centered on the role of the liver in estrogen metabolism, other organs in the intact animal have not been excluded. In fact, in clinical endocrinology the view is often expressed (6–12) that progesterone, acting through the endometrium of the uterus, converts estrone to estriol and thereby exerts a sparing action on the destruction of the estrogens. Thus an elevated recovery of exogenous urinary estrogens and a high ratio of estriol to estrone should characterize the progestational phase of the menstrual cycle in women. Any test of this hypothesis with massive doses

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This work was supported in part by a grant from The American Cancer Society on recommendation of the Committee on Growth.

TABLE 1. EXOGENOUS URINARY ESTROGEN EXCRETION DATA FOLLOWING THE INJECTION OF 2.0 MG. OF ESTRONE IN THE PREOVULATORY AND POSTOVULATORY PHASES OF THE MENSTRUAL CYCLE OF 10 HEALTHY WOMEN
(O = Estrone; D = Estradiol; T = Estriol)

Case	Preovulatory					Postovulatory				
	Total percentage recovery	Distribution pattern			Ratio T/O	Total percentage recovery	Distribution pattern			Ratio T/O
		O	D	T			O	D	T	
1-A*	15.3	44	24	32	0.71	7.9	58	27	15	0.26
1-B	9.6	16	57	27		9.9	9	59	32	
2-A	4.8	40	42	18	0.47	4.4	64	25	11	0.18
2-B	6.4	5	69	26		8.6	13	62	25	
3-A	4.8	37	35	28	0.77	4.3	47	32	21	0.45
3-B	12.3	5	63	32		8.4	3	68	29	
4-A	4.3	46	44	10	0.20	2.0	70	30	0	0.0
4-B	6.4	8	73	19		7.3	10	82	8	
5-A	2.5	64	24	12	0.19	4.6	37	39	24	0.67
5-B	5.2	0	63	37		11.3	16	55	29	
6-A	2.3	61	13	26	0.43	4.0	55	25	20	0.36
6-B	3.3	15	79	6		10.0	16	56	28	
7-A	4.4	57	27	16	0.28	4.7	60	36	4	0.07
7-B	3.2	6	78	16		6.2	21	68	11	
8-A	4.7	49	36	15	0.39	4.0	62	0	38	0.59
8-B	9.1	25	57	18		4.3	7	49	44	
9-A	4.1	54	27	19	0.36	4.5	71	29	0	0.0
9-B	5.4	6	74	20		10.7	33	58	9	
10-A	3.0	50	10	40	0.83	5.3	59	26	15	0.26
10-B	9.6	1	53	46		8.5	11	54	35	
Average—A	5.0	50	28	22		4.6	58	27	15	
Average—B	7.1	9	67	25		8.5	14	61	25	

* A—Simple hydrochloric acid hydrolysis.
B—Zinc-hydrochloric acid hydrolysis.

of the natural estrogens is open to the criticism that such overdosage tends to disrupt normal physiologic processes. We have observed, however, that a single therapeutic dose (2.0 mg.) causes no alteration in the menstrual rhythm, *i.e.*, ovulation and length of cycle. It has seemed appropriate, therefore, to extend our studies of the metabolism of single therapeutic doses of the natural estrogens in human subjects (13, 14) to include studies of the preovulatory and postovulatory phases of the menstrual cycle in healthy women. Although these studies were made primarily to establish a basis for work of a similar nature in women with cancer of the reproductive tract, the data should prove of broader significance to the field of clinical endocrinology, since they bear on the question—Is estrogen metabolism significantly altered in the progestational phase of the menstrual cycle?

The present report, therefore, concerns exogenous estrogen (estrone) metabolism studies in the preovulatory and postovulatory phases of the menstrual cycle in 10 healthy women.

METHODS

A dose of 2 mg. of estrone in oil was injected intramuscularly into each of 10 healthy women (technicians) on the eighth and on the twentieth days[1] of their menstrual cycles. The 48-hour preinjection and postinjection urinary estrogen excretion patterns (estrone, estradiol, estriol) were then determined by our procedure (15) for the chromatographic fractionation and photometric estimation of urinary estrogen. A color correction equation (16) for the Kober reagent was utilized to calculate the estrogen content of each chromatographic fraction. The preinjection urinary estrogen titers were utilized to correct (by subtraction) for the estrogen probably contributed from endogenous sources. Aliquots of each 48-hour specimen were hydrolyzed by hydrochloric acid (10 vols. per cent concentrated hydrochloric acid and twenty minutes reflux) and by zinc dust-hydrochloric acid (15 vols. per cent concentrated hydrochloric acid, 4 Gm. per cent zinc dust, and three hours reflux) hydrolysis. Concomitant pregnanediol determinations by a chromatographic procedure were made on each of the 48-hour urine specimens in order to elicit the presence of a functional corpus luteum (17).

RESULTS AND DISCUSSION

All 10 cases here reported yielded negative results with pregnanediol tests in the urinary specimens collected from the sixth to ninth days of the cycle and positive results with pregnanediol tests in the urinary specimens collected during the eighteenth to twenty-first days of the cycle.

The exogenous urinary estrogen recovery data in Table 1 are expressed

[1] The first day of the flow was considered as the first day of the cycle.

in per cent in order to make certain interrelationships more obvious. Total estrogen data are derived by summation of estrone, estradiol, and estriol titers. Although there is a wide range (2.3–15.3 per cent) in total estrogen recovery in the 10 subjects of this investigation, all responded with increased estrogen excretion during the 48-hour postinjection period and the increment of this excretion over that observed for the 48-hour preinjection period is presumed to arise from the injected estrone. Greater recovery of total estrogen in the postovulatory than in the preovulatory phase of the cycle resulted in 5 cases studied by simple acid hydrolysis and in 7 cases studied by zinc-hydrochloric acid hydrolysis. However, the averages by simple hydrolysis (preovulatory 5.0 per cent, range 2.3–15.3; postovulatory 4.57 per cent, range 2.0–7.9) and by zinc-hydrochloric acid hydrolysis (preovulatory 7.1 per cent, range 3.2–9.6; postovulatory 8.5 per cent, range 3.2–9.6) are similar and do not lend support to the concept that the hormone of the corpus luteum exerts a sparing effect upon estrogen destruction.

Although the view (18) that a progestational endometrium is necessary for conversion of estrone to estriol is no longer tenable, since conversion occurs in men (3–5) as well as in women, its participation in this process has not been excluded. Analysis of our fractionation data in the preovulatory and postovulatory phases of the menstrual cycle shows that unconverted estrone accounted for slightly more than half of the total estrogen recovered in both phases of the cycle (simple hydrolysis averages—preovulatory 50.2 per cent, range 37–64; postovulatory 58.2 per cent, range 37–71), whereas estriol dropped from an average of 21.6 per cent (range 10–40) of the total estrogen in the preovulatory phase to 14.8 per cent (range 0–38) in the postovulatory phase of the cycle. Although this decrease is not statistically significant, it stands in contrast to the concept that conversion of estrone to estriol is more marked in the postovulatory than in the preovulatory phase of the cycle.

The addition of zinc dust during hydrochloric acid hydrolysis of human urine (19) has been shown to yield maximal recovery of exogenous urinary estrogens (12, 20, 21). Furthermore, the elimination by this procedure of pigments which arise in the course of simple hydrochloric acid hydrolysis results in urinary estrogenic residues which produce more typical Kober colors. We have utilized this hydrolytic process on an aliquot of each urine specimen in order to obtain additional information regarding the effect of the hydrolytic process upon the nature of urinary estrogen recovery. It is to be emphasized that the addition of zinc dust during hydrochloric acid hydrolysis of urinary estrogens also causes reduction of the ketonic estrogen, estrone, so that a true picture of the estrogen excretion pattern cannot be obtained by this procedure.

The relatively low (preovulatory 8.5 per cent, postovulatory 13.9 per cent) estrone moieties of our zinc-hydrochloric acid hydrolysates are in line with this observation. The estradiol fraction, which contains the reduced estrone as well as estradiol of metabolic origin, forms approximately two thirds (preovulatory 66.6 per cent, range 53–78; postovulatory 61.1 per cent, range 54–68) of the total estrogen excretion for both phases of the menstrual cycle. There is a similar relative constancy of estriol moieties (preovulatory 24.8 per cent, range 6–46; postovulatory 25 per cent, range 8–44) in the two menstrual phases, so that these data derived by zinc-hydrochloric acid hydrolysis are in agreement with those derived by simple hydrochloric acid hydrolysis and thereby support the observation that there is no consistent distinguishing pattern of exogenous estrogen excretion for either phase of the menstrual cycle.

All except 2 of the 20 urine specimens yielded increased total exogenous urinary estrogen titers by addition of zinc dust during hydrolysis. However, only 3 (Nos. 3, 8, and 9 in the Table) of the 10 cases showed a greater increment of estrogen recovery in the presence of zinc during the preovulatory phase than during the postovulatory phase of the cycle. The average increment for the postovulatory period (3.9 per cent) was in fact greater than that for the preovulatory period (2.0 per cent). These data therefore are at variance with the concept of the Smiths (11) that there is a greater increment of estrogen recovery by zinc-hydrochloric acid hydrolysis over simple hydrochloric acid hydrolysis during the preovulatory than during the postovulatory phase of the menstrual cycle. It should be emphasized that our data were obtained colorimetrically in contradistinction to the bioassay techniques of the Smiths and that the data, therefore, might not be strictly comparable.

CONCLUSIONS

Exogenous urinary estrogen excretion patterns (estrone, estradiol, estriol) of 10 healthy women following injection with 2.0 mg. of estrone in the preovulatory and postovulatory phases of the menstrual cycle were strikingly similar, so that no consistent distinguishing pattern for either phase was observed. From these observations one is forced to conclude that the physiologic status (proliferative or progestational) of the endometrium in women contributes so feebly, if at all, to the character of urinary estrogen excretion pattern that no dependable deductions as to physiologic status of uterine endometrium can be derived therefrom.

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THE ABILITY OF THE HUMAN LIVER TO REDUCE THE BIOLOGIC ACTIVITY OF INJECTED ESTROGENS*

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THE role of the liver in the metabolism of estrogens has been the focus of numerous studies which have been recently reviewed by Pearlman (1) and by Glass (2). Incubation of estradiol with liver slices, liver homogenate or cell-free extracts of liver tissue effects a marked reduction in biologic activity of the incubated estrogen (3, 4, 5, 6). Conversely, various types of experimental and clinical hepatic injury have been shown to be associated with evidence of increased biologic activity of exogenous or endogenous estrogens (2). Golden and Sevringhaus (7) have shown that the estrogen produced by an ovary transplanted into the drainage area of the portal vein in the rat is biologically ineffective. However, Hooker *et al.* (8) reported that intrasplenic administration of estrogens in the monkey is not associated with reduced biologic effect. Similarly, Van Wagenen and Gardner (9) observed no impairment of menstrual function in the monkey when the ovaries were transplanted into the spleen. Thus the evidence derived from primates conflicts with that obtained in rodents and presents some doubt regarding actual estrogen inactivation in the human liver.

Our interest in the problem has arisen from a study of the therapeutic application of steroid hormones in patients with breast and prostate cancer (Hertz *et al.* (10)) and from a consideration of the possible role of hepatic impairment in relation to steroid activity in congestive heart failure (Evans *et al.* (11)).

MATERIALS AND METHODS

The subjects for this investigation have been patients with inoperable breast or prostate cancer admitted to the hospital for evaluation of the clinical effect of intensive parenteral estrogen therapy. Their nutritional

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state varied from very poor to good. The condition of the liver was appraised by history, physical examination and the series of liver function tests shown in Table 1. Only those patients who were free of any evidence of liver disease are included in this report. Nevertheless, the presence of cancer in all of these subjects may have been a factor in determining the effects observed.

TABLE 1

Initals, age and sex	Diagnosis	B.S.P. (% ret'd. 45 min.)	Total serum bilirubin (mg. %)	Cephalin flocc. (24/48 hrs.)	Thymol turb. (units)	Serum proteins: alb./glob. (grams %)	Oxygen determinations			
							H.V.† (% sat.)	F.A.-H.V.: diff. (vol. %)	Cap. (vol. %)	F.A.‡ (% sat.)
I. M.R. 69 M	Ca breast Ca prostate	0	0.2	0/+	1.3	3.4/4.3	56	5.1	12.8	92
II. V.S. 43 M	Ca breast	22*	0.7	+/++	2.0	3.4/2.5	54	5.8	13.5	97
III. A.S. 89 M	Ca prostate	0	0.5	0/++	2.5	3.4/3.1	52	6.8	15.2	94
IV. R.E. 41 F	Ca breast	0	0.4	0/0	2.0	4.2/2.5	60	5.3	15.1	94
V. C.G. 56 M	Ca prostate	0	0.5	0/0	6.3	3.6/3.1		6.9		
VI. M.S. 50 F	Ca breast	0	0.3	0/0	7.5	1.9/4.5	32	8.2	16.7	81
VII. B.R. 73 F	Ca breast	0	0.8	—	5.0	3.1/3.0	55	7.0	15.1	99
VIII. E.T. 67 F	Ca breast	0	0.5	0/0	6.0	2.7/3.4	41	7.0	12.9	90
IX. G.G. 56 F	Ca breast	0	0.3	0/?	2.0	3.4/2.7	64	5.0	16.7	95

* Initial B.S.P. retention in this case was due to the transient effect of previously administered estrogens; this phenomenon is to be fully described in a separate report.

† H.V.—Hepatic vein.

‡ F.A.—Femoral artery.

Under moderate Amytal sedation and Demerol analgesia, a cardiac catheter was inserted via an antecubital vein into the right hepatic vein under fluoroscopic observation. A Cournand needle was introduced into a femoral artery and left in place for periodic withdrawal of peripheral blood. After control samples of blood were obtained, a solution containing a mixture of water-soluble conjugated equine estrogens,[1] or a solution of water-soluble synthetic potassium estrone sulfate,[2] was administered intravenously at a carefully controlled rate varying from 0.5 to 2.0 mg. per minute. Blood samples of 20 ml. each were taken as nearly simultaneously as possible from the hepatic catheter and the femoral artery at approxi-

[1] "Premarin" Injectable supplied by Ayerst, McKenna & Harrison Ltd. through the courtesy of Dr. E. C. Reifenstein, Jr.

[2] Potassium Estrone Sulfate supplied by the Research Laboratories of the Schering Corporation.

mately ten-minute intervals for forty to seventy minutes. The blood was allowed to clot and the serum assayed for estrogenic activity. The catheter was carefully cleared of saline by removing and discarding 5.0 to 6.0 ml. of blood prior to obtaining the test sample. In 3 patients, duplicate samples were taken in heparin and determinations made upon plasma and whole blood. In 5 instances samples were taken simultaneously from the hepatic vein, the femoral artery and a peripheral vein. There was no significant difference in the estrogenic activity of blood from the femoral artery and a peripheral vein, or between plasma and serum. All sampling was performed under stop-watch guidance. The time of each sample was recorded to the nearest ten seconds and in those instances in which sampling was not simultaneous, the time was recorded individually and graphed accordingly. Plasma volume determinations were made during the procedure, by the Evans Blue T-1824 method. In 3 patients blood samples were drawn three, six and nine minutes after injection of the dye. This was done to compare the "mixing time" of the injected materials at the various sites of blood collection and to exclude the possibility of saline dilution of the catheter sample. A Beckman model DU spectrophotometer was employed for the T-1824 determinations. These sets of samples showed no significant difference in dye concentration in femoral artery and hepatic vein samples. Blood samples were drawn simultaneously under oil from the hepatic vein and from the femoral artery and analyzed for their oxygen content by the method of Van Slyke and Neill (12). At the end of the procedure the position of the catheter tip was verified by a roentgenogram.

Estrogen assays on serum, plasma, and whole blood were performed by a modification of the rat uterine growth method of Lauson *et al.* (13). Results are expressed as gamma per cc. of estrogenic activity, using the injected estrogen preparation as a reference standard. This is a measure of over-all biologic activity only, and yields no precise information as to either the quantity or the chemical nature of the estrogenic complex present in the serum.

The serum bromsulphalein level was determined forty-five minutes after the injection of 5.0 mg. per kilogram, by the method of Mateer *et al.* (14). The bilirubin concentration was determined by the method of Malloy and Evelyn (15); the cephalin-cholesterol flocculation, by the method of Hanger (16); the thymol turbidity, by the method of Shank and Hoagland (17); and the serum albumin and globulin, by the method of Cohn and Wolfson (18).

RESULTS AND DISCUSSION

The diagnosis, nutritional evaluation, and liver function tests are shown in Table 1 for each of the 9 patients.

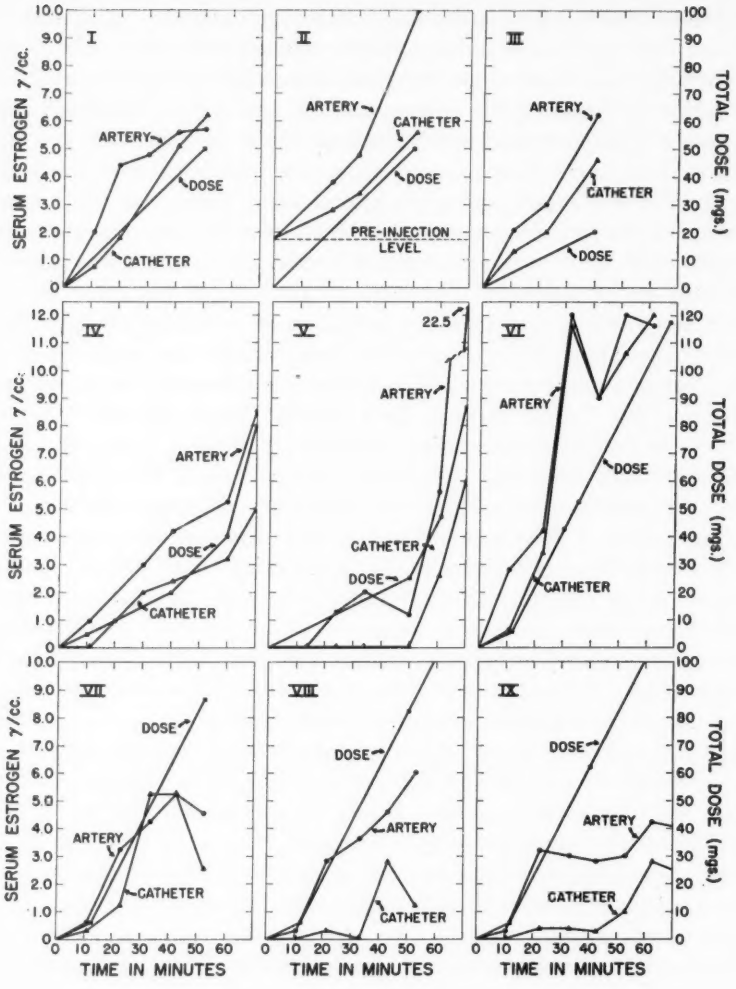

PLATE 1

Comparative estrogen activity in peripheral artery and hepatic vein.
Numerals correspond to cases shown in Table 1.
In Cases I through V the patients received conjugated equine estrogens and in Cases VI through IX they received crystalline potassium estrone sulfate.

The percentage oxygen saturation and the difference in oxygen content between the hepatic vein and the femoral artery are also shown in Table 1. The normal value for the hepatic vein oxygen saturation is 60 per cent or greater. The normal difference between the oxygen content of the hepatic

vein and femoral artery does not exceed 4.0 volumes per cent. The hepatic vein oxygen saturation in our cases ranges from 32 per cent to 60 per cent, indicating in all instances moderate to marked hypoxia. The arterial-hepatic venous oxygen difference was increased in many instances, ranging from 5 to 8.2 volumes per cent. The relative anoxemia may have been on the basis of the degree of sedation during the procedure, or may reflect alterations due to the basic disease process. In 1 instance determinations of the arteriovenous oxygen difference before and after the infusion of estrogens failed to show any significant alteration. There is no readily apparent correlation in this small group of subjects between the degree of hypoxia and the ability to alter serum estrogen activity by circulation through the liver, although the patient M.S., with the lowest hepatic vein oxygen saturation (32 per cent) showed relatively poor inactivating capacity.

The results of the estrogen bioassays are presented in individual graphs (Plate 1) for each patient. The estrogenic activity of serum from the hepatic vein and that of serum from the femoral artery are plotted in terms of gamma per ml. on the left ordinate, and the total dose of estrogen in mg. of activity on the right ordinate. Time in minutes from the beginning of the infusion is shown on the abscissa.

The estrogenic activity of the serum obtained from the hepatic vein was lower than that from the femoral artery in each of the 9 patients studied. In 3 patients (I, VI, IX) the differences obtained after infusion of 40 mg. of estrogen fell within the range of error of the assay, which averaged 15 per cent throughout these studies. All 3 of these patients weighed less than 50 Kg. and were in a poor state of nutrition. Patient VI, who showed only limited evidence of estrogen inactivation, was nutritionally depleted and had diffuse pulmonary metastases, reflected in the arterial oxygen saturation of 81 per cent. The remaining 6 patients exhibited a consistent and significant difference between hepatic vein and femoral artery activity throughout the range of doses administered.

In view of the known disparity in the relative biologic activity of various forms of estrogen such as estrone, estradiol, and estriol, which are formed *in vivo* following the injection of estrone (1), a quantitative estimate of the amount of estrogen removed by the liver under the conditions of these studies would not be valid. It should be pointed out that use of the femoral artery sample as representative of portal inflow assumes that the estrogen content of arterial blood is not significantly altered in its passage through the capillary bed of the portal drainage area. The absence of significant variation in the estrogen titer of simultaneous samples from the femoral artery and a peripheral vein provides evidence that estrogen apparently remains unchanged in its passage through the peripheral capillary bed.

However, further observations, with direct sampling of the portal vein, are needed to establish the validity of the use of the femoral artery sample.

It is apparent in certain of our patients that the metabolic capacity of the liver for estrogen may be extensive, as shown by continued "inactivation" as the infusion dose was progressively increased. The blood levels of estrogen attained in this study exceed by far the concentrations previously observed under physiologic conditions, including normal pregnancy. It should be pointed out, however, that the estrogens infused are in a different biologic state from the endogenous estrogens (19).

The hepatic mechanisms acting to reduce the activity of circulating estrogen are probably multiple. Cantarow and associates (20) have demonstrated excretion of a major portion of injected estrogens in the bile, and have postulated an enterohepatic circulation of estrogens similar to that established for bile salts. If the major portion of injected estrogens is excreted in the bile and subsequently returns to the liver, other factors must be at work to "inactivate" the estrogens that are resorbed. It is also possible that a certain amount of the reduction of the biologic activity of injected estrogens described here may be due to transient storage within the liver.

SUMMARY

1. A procedure is described for study of the hepatic metabolism of steroids in man by hepatic vein catheterization.

2. Observations were carried out in 9 patients, with normal liver function, receiving large amounts of water-soluble estrogens intravenously in connection with therapy of cancer of the breast or prostate. As determined by bioassay, estrogenic activity was less in blood leaving the liver (hepatic vein) than in blood entering the liver (femoral artery).

3. The limitations imposed on interpretation of the results and the mechanisms whereby hepatic "inactivation" may take place are discussed.

Acknowledgment

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PERSONALITY CHANGES IN CUSHING'S SYNDROME*

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IN THE past five years there has been an increasing number of reports describing mental disturbances precipitated by treatment with ACTH and cortisone (1, 2). Another sequel of administering excess ACTH is the occurrence of the typical Cushing's syndrome (3). Thus there arises the question whether the hormonal changes that are responsible for this syndrome may not also represent a chemical basis for the psychiatric changes.

Since the original description by Dr. Cushing in 1932 (4), various authors have recognized that mental disturbances are frequent concomitants of the syndrome. Many papers make brief or casual reference to extreme depression and dullness. In the past, however, these emotional alterations have almost always been attributed to poor social adjustment secondary to the often repulsive appearance of these patients. The mental symptoms were regarded as nonspecific affective disorders which were interpreted as the result of self-consciousness. Maclay and Stokes (5) and Schlesinger and Horwitz (6) were among the first to suggest specificity in the relation between the psychosis and endocrine dysfunction in this syndrome.

Now that we know that the hormone (ACTH) may, in some patients, produce Cushing's syndrome and may also induce psychotic episodes, a re-evaluation of these relationships is in order. It is the purpose of this paper to record the relevant findings gleaned from a scrutiny of 53 proven cases of Cushing's syndrome and to determine the incidence and nature of the mental and emotional aberrations.

Most of these cases have been reported elsewhere (7). In the earlier report, however, no specific reference was made to psychotic disturbances except when suicide was the cause of death. It is noteworthy that personality changes were present in 60 per cent of these cases. They existed regardless of the basic pathology. There were 39 pituitary adenomas, 7 adrenal tumors, 6 cases showing hyalinization of anterior lobe basophils (Crooke's change) without tumor, and 1 arrhenoblastoma with Crooke's change (Table 1).

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* This material was made available through the kindness of Dr. Louise Eisenhardt, who personally verified the pathology.

TABLE 1. ANALYSIS OF 53 CASES OF CUSHING'S SYNDROME

Personality changes	Pituitary adenoma	Adrenal tumor	Crooke's change without tumor	Arrhenoblastoma	Total
Mental changes requiring isolation	6	3	3	1	13
Severe or moderately severe	14	4	1	0	19
Normal or minimal	19	0	2	0	21
Total number of cases	*39*	*7*	*6*	*1*	*53*

Severe depression was the most frequent finding. Euphoria, which often occurs with ACTH therapy (8, 9), was a rare finding in this series. Figure 1 illustrates the frequency of significant mental or emotional disturbances in these proven cases. Thirteen (24.5 per cent) of the patients showed such severe personality disturbances that 8 were institutionalized as insane and 5 others attempted suicide. An additional 19 patients (35.8 per cent) had marked or moderate mental symptoms but isolation was not necessary. A recent paper reports a cortisone-induced suicide in a previously normal person (10). The suicide rate in our series is 1,000 times that of the general population.

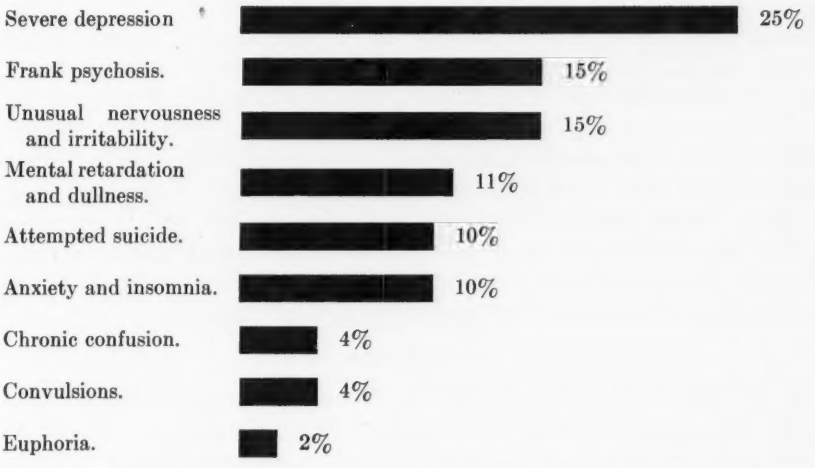

FIG. 1. Graph showing the frequency of significant mental disturbances in Cushing's syndrome.

COMMENT

It is apparent that the term Cushing's syndrome encompasses several pathologic entities producing the same symptom complex. Although the most constant histologic finding is the presence of hyalinized basophils in the anterior lobe of the pituitary (Crooke's change), Albright believes that the pathologic physiology is essentially an adrenal hormone metabolic dysfunction (11). The symptoms are manifestations of excess adrenocortical substances, even in those cases in which all of the demonstrable pathology is elsewhere (*i.e.*, in the pituitary). Adrenal steroids have been implicated as the source of the various symptoms. The adiposity is believed to be the result of increased 11-dehydrocorticosterone (compound A of Kendall) or allied substances. The alterations in the potassium, chloride and water balance are caused by excess of the desoxycorticosterone group. These changes produce muscle weakness, periodic paralysis, hypertension, edema, cardiac dilatation, osteoporosis, and thin skin with striae. Hirsutism, amenorrhea, acne, and erythrocytosis are attributed to excess adrenal androgens. The diabetes and other disturbances of sugar and protein metabolism probably result from steroids such as 11-dehydro-17-hydroxycorticosterone (compound E of Kendall, S hormone of Albright) (12).

There is already a considerable mass of evidence that a definite relationship exists between hormones and behavior. In 1934 Harris, Horwitz, and Katz (13) made extensive studies to fathom the nature of this association. In 1945 Pincus and Hoagland (14) found that the response of the adrenal cortex to ACTH in schizophrenic patients was significantly more sluggish than among normal controls. In 1949 Hoefer and Glaser (9) observed that ACTH therapy produced "alterations in mood, affective level, and behavioral activity." Thus it seems apparent that ACTH and adrenal function constitute important links in the connection between hormones and behavior. Numerous other authors have made observations further substantiating this viewpoint (15, 16). The concomitant production of Cushing's syndrome and emotional disturbances by ACTH provides further suggestive evidence for the existence of a chemical basis for certain of the psychoses. Any categorical statement at this time would, of course, be speculating far beyond the range of established facts.

CONCLUSIONS

In 53 verified cases of Cushing's syndrome there was specific mention made of the emotional status of the patient. Many (40 per cent) showed no remarkable mental changes. Thirteen (25 per cent) showed frank psychosis, either necessitating institutionalization or resulting in attempted suicide. An additional 19 (35 per cent) had definite and marked personality altera-

tions. These latter may have been secondary to the overwhelming weakness of the disease or to the often grotesque appearance of the patients. Psychosis occurred with sufficient frequency and severity to be adjudged a significant part of the syndrome. This observation, when considered in the light of recent ACTH studies, suggests that behavior disturbances in many cases are primary manifestations of the syndrome. It is unlikely that ACTH itself is the responsible factor, since euphoria is the most common mental change induced by ACTH but is rare in cases of Cushing's syndrome.

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METABOLIC STUDIES IN GOUT*

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GOUTY arthritis has been classified as a disorder of metabolism ever since Garrod demonstrated hyperuricemia in this disease (1). Until recently, investigation in this field centered around the uric acid content of blood and urine. It has become evident, however, that hyperuricemia alone cannot adequately account for the body alterations seen in clinical gout. For example, most studies (2, 3, 4, 5) fail to demonstrate a specific pattern of change in urate metabolism in asymptomatic gout as contrasted with acute gout. Also, clinical gout does not develop in patients with other diseases accompanied by hyperuricemia. In addition, colchicine, although effective in the treatment of articular gout, has no perceptible influence on uric acid metabolism.[1] Consequently one might assume, as many investigators in this field have done recently, that the hyperuricemia, rather than being primarily causal, may be only a reflection of other more pertinent metabolic disturbances which, if brought to light, might help dispel some of our ignorance concerning this ancient but still enigmatic disorder.

The observation that ACTH and cortisone, in addition to their other metabolic effects, enhanced the urinary secretion of uric acid led to the trial of these hormones in patients with gout, and the favorable results have since been well documented (6–12). Accordingly, the adrenal cortex has now been assigned a position of prominence by investigators in this field. Attention was first directed toward the adrenal cortex in gout by Talbott and co-workers (2, 13). They pointed out that the regularly occurring electrolyte cycle in gout, with the prodromal diuresis of water, sodium and chloride, resembled the findings in adrenal insufficiency. Selye (14) also suggested a possible relationship between the adrenal cortex and gout by classifying gout as one of his "diseases of adaptation." The time-honored

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[1] Recent studies by Bishop *et al.* (43) have suggested that colchicine might decrease the rate of uric acid synthesis.

but previously incomprehensible observation that attacks of acute arthritis in patients with gout frequently follow such unrelated events as surgery, trauma, infection, drug administration and hemorrhage (15–22) is now being meaningfully interpreted in relation to adrenal cortical activity, and the unrelated events are now called "nonspecific stresses." Leblond and Segal (23), using colchicine in rats, came to the conclusion that this drug produces stimulation of the pituitary adrenal axis, from which might be inferred a possible explanation for the efficacy of colchicine in human gout. Recently, Wolfson and co-workers (12, 24) have attempted more definitive elucidation of the possible relationship between the pituitary-adrenal axis and gout and have concluded that measurable temporary hypocorticalism, or lack of heightened response of the adrenal cortex to stress, even during an acute attack, is present in patients with gout. Rather than the primary defect being in the adrenal cortex, they feel that there is less than the normal amount of ACTH released by the pituitary in response to stress in these patients. They also feel that colchicine in therapeutic doses stimulates the pituitary-adrenal system to a slight degree, but that this effect is not sufficiently marked to explain the therapeutic action of the drug. Thus, in spite of some evidence to the contrary (25), all of the foregoing observations suggest that the pathogenesis of gout may be related to a dysfunction of the pituitary-adrenal axis.

This paper describes a study, begun before some of the above data were available, of a small group of patients with gout, with particular reference to the activity of their adrenal cortices during asymptomatic and acute phases, and their response to colchicine, ACTH and testosterone. Metabolic observations were made during a total of 12 attacks of gout in 6 patients. In addition, 10 cases of spontaneous gouty arthritis were observed clinically during therapy as noted later. Tests were performed during the interval phase and during the acute phase for both spontaneous and ACTH-induced attacks of the disease. The effects of colchicine upon adrenal cortical function were studied also during its administration in both the asymptomatic and acute phases of the disease. All patients received an average hospital diet, except for patient F.M. who received an 800-mg. sodium diet.

The subjects were followed with at least daily eosinophil counts and frequent determinations of the excretion of neutral lipid-soluble reducing substances in the urine (hereinafter to be referred to as 11-oxysteroids,[2] although this fraction also contains other substances), sweat and serum

[2] A more accurate definition of this fraction is "reducing corticoids," as suggested by Corcoran (*J. Clin. Endocrinol.* **11**: 445, 1951).

electrolytes, and serum uric acid. In addition, periodic estimations of blood sugar and glutathione levels and daily urinary uric acid measurements were obtained in certain of these cases. Whenever possible, the assessment of basal adrenal-cortical activity[3] was calculated on the basis of the average of several consecutive daily tests.

METHODS

Eosinophil counts were determined by the method of Dunger (26) with modifications suggested by Thorn *et al.* (27). Uric acid was determined by the method of Folin (28), 11-oxysteroids by the method of Heard, Sobel and Venning (29), sweat sodium by the method of Leslie and Levin (30), blood sugar by the method of Kingsley and Reinhold (31), and glutathione by the method of Woodward and Frey (32).

RESULTS

A. *Adrenal-cortical activity in asymptomatic and acute gout*

As noted in Figure 1, the results of the tests showed little change between the asymptomatic and the acute episodes of gout.

Except for J.M., in whom an increase of 110 per cent in eosinophils occurred during the acute phase, possibly suggesting hypocorticalism, there were no significant eosinophil changes found. (It must be emphasized that the inherent errors in the methods used were at least as large as the variations observed in most instances. For example, daily basal eosinophil counts in this group of patients revealed fluctuations of 23, 69, 42 and 40 per cent. It probably is unwise to place undue emphasis on resting eosinophil changes of only moderate degree.)

11-Oxysteroid determinations similarly failed to reveal a significant change during acute attacks. Again the lone exception was J.M.; however, it is felt that the variation of a basal output of 3.17 mg. contrasted with 2.48 mg. per twenty-four hours during his attack is equivocal.

Variations in the sodium content of sweat were also not significant. In no instance was there an appreciably higher excretion of sweat sodium (which might indicate hypocorticalism).

Additional data relating to adrenal cortical activity in acute as compared to interval gout are depicted in Figure 2. In the first 2 recorded patients (A.McG. and J.M.) acute gout developed on the sixth and seventh days, respectively, after ACTH was discontinued. As is apparent, in neither instance was there a significant change in sweat sodium, 11-oxysteroids or eosinophils.

[3] It is apparent that this is not an assessment of whole adrenal-cortical function since most of the tests performed reflect the activity of the so-called "glucocorticoid" fraction only.

Thus, in the patients studied, no important differences in adrenal cortical activity, as measured by the tests performed, were observed between acute gouty arthrititis and interval gout.

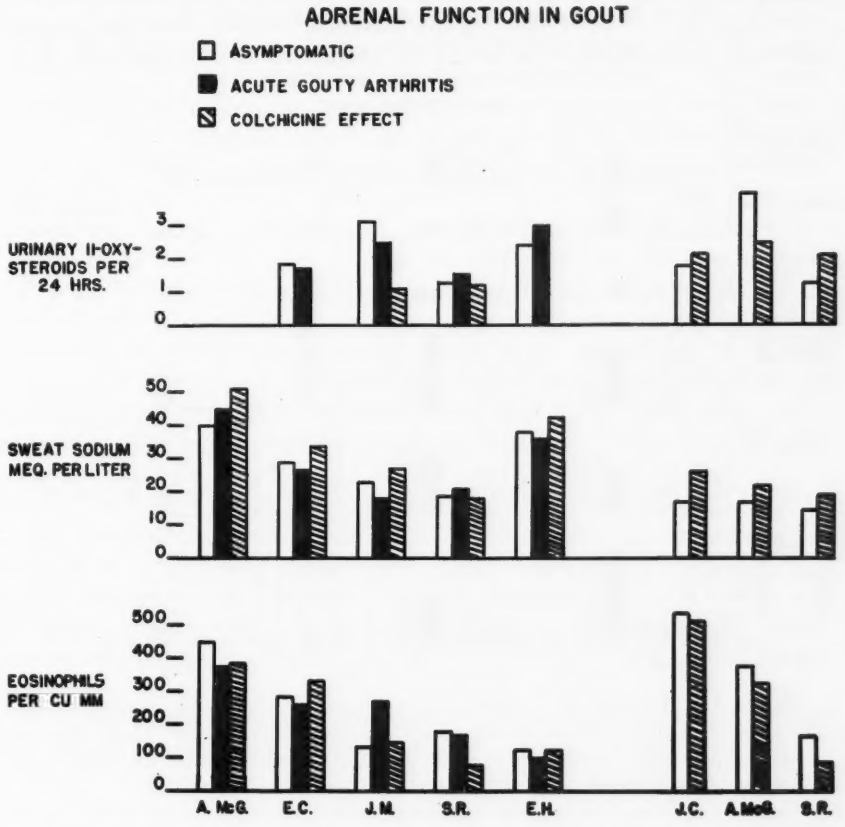

FIG. 1. *Note:* 1. Relative consistency of adrenal cortical function in the interval and in the acute stage of the disease. 2. Colchicine produces no effects consistent with increased adrenal cortical activity when given during acute stage or while patient is asymptomatic.

B. *The effect of colchicine upon the pituitary-adrenal axis*

Figures 1 and 2 depict the effects of colchicine. As is apparent in the graphs, in 5 patients a total of 5 spontaneous and 2 post-ACTH attacks were treated and studied. In addition, 3 of these patients were studied during the administration of colchicine, while asymptomatic. The drug was administered in 0.5-mg. oral tablets. Each patient received hourly

doses to the point of tolerance as judged by therapeutic response, gastrointestinal reactions, or both. During the 24-hour period studied, ten to seventeen doses were ingested. All of the patients who received colchicine during their acute gout experienced prompt subjective and objective relief.

During the 7 treated attacks, the eosinophil counts were lowered significantly in 2 instances—patients J.M. and S.R. These changes were from

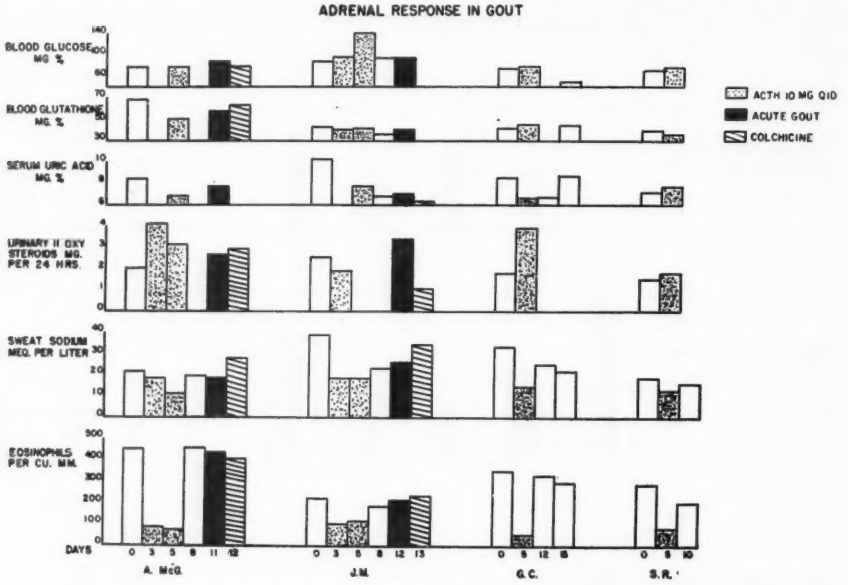

FIG. 2. ACTH was given for five days in all cases, during the asymptomatic stage. The clear blocks at the start of each study represent average basal values. The clear blocks depicted later demonstrate the data during the post-ACTH period, while the patients were asymptomatic.

275 to 138 and from 175 to 85 eosinophils per cu. mm., respectively (Fig. 1). However, when 1 of these 2 patients (J.M.) was given colchicine a second time for the attack which occurred after ACTH was discontinued (Fig. 2), eosinopenia failed to develop again. As is apparent in the data, in neither of these 2 patients is there collateral evidence, as measured by sweat sodium or urinary 11-oxysteroid output, of increased adrenal cortical activity.

The effect of colchicine in 3 patients studied during the asymptomatic phase of their disease is shown on the right side of Figure 1. Data on the first 2 cases (J.C. and A.McG.) revealed no evidence of increased adrenal cortical activity. Patient S.R. showed some changes compatible with mild

stimulation of the adrenal cortex. His eosinophils decreased from 157 to 88 per cu. mm. and his urinary 11-oxysteroids increased from 1.27 to 2.11 mg. per twenty-four hours. However, the change in the sodium content of his sweat did not correlate with these findings, increasing from 14.4 to 19.1 mEq. per liter. (It is noteworthy that in measuring sweat sodium during colchicine administration in this study, there was a slight to moderate increase in 9 out of 10 determinations. The explanation is not apparent).

Of particular interest are the uncharted observations in 1 patient (E.C., Fig. 1), who, during an attack of gout, ingested 48 self-administered tablets of 0.5 mg. of colchicine in a period of thirty hours. He exhibited rather profound evidence of colchicine intoxication, with severe vomiting and diarrhea during the first day of hospitalization. At this time, his eosinophil count was 53 per cu. mm. Several days later, when asymptomatic, his count stabilized in a range of 269 to 313. This suggests that adrenal cortical activity can be augmented significantly in patients with gout if the stress is powerful enough.

It appears justifiable to conclude from the data described that colchicine, in therapeutic dosage, had no measurable effect on the pituitary-adrenal systems of the patients studied.

C. *Metabolic response to ACTH*

The metabolic response to ACTH in 4 patients with gout is represented in Figure 2. In each case, a dose of 10 mg. of Armour's ACTH was injected intramuscularly every six hours for five days. Studies were continued throughout the immediate post-treatment period, either until there was an exacerbation of acute arthritis, or until it was evident that none would occur.

A definite eosinopenia was produced in each case. The level of sodium in the sweat was significantly lowered in all cases, and in all but 1 patient (J.M.) the concentration of sweat sodium fell to an abnormally low level (below 15 mEq./L.). The 11-oxysteroid excretion increased significantly in 2 cases (A.McG. and G.C.), slightly in 1 (S.R.) and fell slightly in 1 (J.M.). The serum uric acid concentration decreased in 3 of the 4 patients during administration of ACTH. Significant elevation of the fasting blood sugar was noted in only 1 case (J.M.). A constant inverse relationship as described by Conn (33) between glutathione and blood sugar was not observed—J.M.'s blood sugar rose without a simultaneous significant fall in his glutathione level. Conversely, in the case of A.McG., a definite lowering of glutathione was noted without much change in his fasting blood sugar.

Uncharted are the observations on the serum electrolytes, which were normal throughout the study. Also, daily urinary uric acid determinations

on A.McG. while receiving ACTH revealed a marked increase in the amount excreted. His average basal excretion was 2.02 Gm. per twenty-four hours and this value rose to 3.04 Gm. during the period of treatment.

The over-all response to ACTH appeared grossly to be within the accepted normal range. In some instances the changes noted were suboptimal, which may be explained by the relatively small doses used and individual variations in responsiveness, rather than by abnormal adrenal cortical function.

The data on A.McG. and J.M. seem noteworthy. In these 2 patients, symptoms of acute gout developed six and seven days, respectively, after discontinuance of ACTH. At the time arthritis developed, their adrenal cortical activity as measured by the tests performed, was approximately at the same level as before treatment. None of the data obtained at the time of the post-ACTH attacks pointed to hypoadrenal cortical activity, *i.e.*, there was no increase of eosinophils or sweat sodium and no decrease of 11-oxysteroids. Patients G.C. and S.R., although their data are less complete, showed essentially the same type of adrenal response after ACTH was withdrawn, *i.e.*, a return of adrenal cortical activity to the pretreatment range. In neither of these patients, however, did gout develop when this level was reached. Therefore, it would seem that the development of post-ACTH arthritis in the gouty patients studied was not related to the level of adrenal cortical activity as assessed by these tests.

D. *Effects of testosterone*

The observations during the administration of testosterone propionate to these patients were not charted. This steroid was given intramuscularly in doses of 50 to 100 mg. per day for eight to ten days to the same 4 patients who received ACTH. Using the same tests, no consistent significant metabolic alterations were observed. In 3 of these 4 patients an acute attack of gout developed from two to five days after testosterone was withdrawn. The only patient in whom a post-testosterone attack did not develop was J.C., a man with chronic tophaceous gout.

Ten other patients with acute gouty arthritis were then treated with testosterone propionate intramuscularly in doses of 100 to 200 mg. per day for three days. Aspirin and colchicine were withheld. Of this group, 2 showed no improvement. (One of these was a man who had also previously failed to respond to 100 mg. of ACTH; when treated with colchicine, he responded satisfactorily.) Three patients manifested slight subjective improvement. In 5 of the 10, subjective and objective improvement was definite, although not quite as rapid as that usually seen with colchicine. (However, 1 of these patients had been treated with colchicine previously and he felt that his response to testosterone was faster and more satis-

factory than that afforded by colchicine.) Of the 5 patients who responded well, 2 had an acute exacerbation within several days after discontinuing therapy. Since the patients with a minor or no effect after testosterone were subsequently given colchicine, the development of a post-testosterone exacerbation was impossible to judge.

In summary, testosterone in daily dosage of 100–200 mg. relieved satisfactorily 5 out of 10 attacks of acute gout. Among the 5 patients who obtained relief, in 2 an exacerbation developed within a few days. In 3 of the 4 patients who received testosterone during the asymptomatic stage, there was a flare-up after this drug was discontinued.

E. *Tophi*

One of our patients (J.C.) was suffering from chronic tophaceous gout and had previously undergone amputation of several digits because of tophaceous infiltration. He was admitted to the hospital with large subcutaneous masses on his hands and feet, which on biopsy were found to contain uric acid crystals; two draining sinuses on his feet also contained urate crystals. During two courses of ACTH (40 mg. per day for five days and a second course for thiry-one days) a definite reduction in the size, inflammation and firmness of several of his tophaceous masses was noted. The draining sinuses did not change. Courses of cortisone and testosterone (100 mg. for seven and eight days, respectively) produced similar results, although benefit from cortisone appeared less than from ACTH, and testosterone was even less marked in its effect. Within a few days after discontinuing therapy the tophi slowly returned to their pretreatment status. This experience is approximately the same as that previously reported by Thorn *et al.* (7).

DISCUSSION

In acute gout, administration of a physiologic excess of ACTH is usually followed by prompt improvement. In attempting to interpret this phenomenon, it has been suggested by Wolfson and co-workers (12, 24) that gout is, in part, an adrenal cortical deficiency disorder, secondary to a decreased release of ACTH in response to stress. This is based primarily on their observation of a lack of increased adrenal cortical activity in the acute as contrasted with the interval phase of this disease.

Some of our data are similar to those of the aforementioned investigators, in that our patients demonstrated no evidence of increased adrenal cortical function during acute gout, and their response to exogenous ACTH was within the normal range. However, rather than interpreting these findings as pointing to a dysfunction of the pituitary-adrenal system, other explanations seem worthy of consideration: 1) An acute attack of

gout may not be sufficiently stressful to stimulate the pituitary-adrenal axis. In support of this are the findings in patient E.C. He showed no evidence of augmented adrenal cortical function during his acute attack of gout, but after intoxicating himself with colchicine, a definite eosinopenia was apparent. 2) Many metabolic actions that are being attributed to the adrenal cortical hormones may in reality be due to other, still unknown, regulatory mechanisms. As emphasized by Ingle *et al.* (34) and Herlant (35), although the metabolic responses to stress cannot occur in the absence of adrenal cortical hormones, they probably are not produced by these hormones. It may be that the primary function of these hormones during stress is to make the organism more sensitive to the action of other substances. Accordingly, it is possible that the measurable metabolic changes usually evident as a result of stress are absent in patients with gout primarily because of dysfunction of these obscure regulatory mechanisms rather than because of pituitary-adrenal deficiency.

Certain information derived from this study seems to cast doubt upon the concept that the pituitary-adrenal system plays a central role in the pathogenesis of gout. Colchicine, according to the data presented, had no demonstrable effect on the pituitary-adrenal systems of the patients studied. This drug is an effective therapeutic agent, not only in the treatment of spontaneous attacks but also in the prophylaxis of post-ACTH and post-surgical gout (12, 17). If one assumes that the acute episodes, particularly in these latter instances, are primarily related to adrenal cortical deficiency, one should reasonably expect an agent as specifically beneficial as colchicine to have some influence on pituitary-adrenal function. Testosterone, which appears to possess some therapeutic value in the treatment of acute gout, also did not measurably affect adrenal cortical activity in our patients. This hormone is a pituitary inhibitor rather than a stimulator (36–38).

The acute exacerbation of gouty arthritis that follows the discontinuance of exogenous ACTH administration is explained by Wolfson and others (12, 24) on the basis of the temporary inhibition of endogenous ACTH release. As observed in this study, the withdrawal of testosterone, which in large doses may likewise inhibit ACTH release (36–38), seems also to be followed by acute gouty arthritis in some patients. On the other hand, as Gutman and Yu have emphasized (25), disorders characterized by pituitary or adrenal deficiency, as Simmonds' disease and Addison's disease, are not associated with gout. Even if one assumes that patients with gout are hereditarily unique and in them alone acute manifestations of the disease develop with decreased pituitary-adrenal activity, the data in this study are not supportive; not only the spontaneous but also the post-ACTH attacks occur at a time when adrenal cortical activity, as measured

by the tests performed, is at basal, not subnormal levels. The explanation for the post-ACTH exacerbations of gout is still obscure, in our opinion.

At the present time, the tests available for studying pituitary-adrenal activity on a clinical level are still crude, and it is possible that when more specific methods become available, one may be able to demonstrate a precise relationship between some phase of adrenal cortical function and the patho-physiology of gout. Until such time, the role of the pituitary-adrenal system in gout probably will remain speculative.

In the light of our present imperfect knowledge, the prompt improvement of acute gouty arthritis following ACTH administration and the post-ACTH exacerbation may be more crudely but plausibly explained on the basis that the hormone has a nonspecific antiphlogistic effect (39), and that discontinuance of the hormone permits the attack to recur (25). The eventual elucidation of the action of colchicine, which appears specifically to counteract the acute attack rather than merely suppress it (since its withdrawal is not followed by a recurrence), will undoubtedly be richly rewarding.

It is worthy of emphasis that the tests used in this study measure primarily "gluco-corticoid" activity, since even the electrolyte content of serum and sweat are influenced to some extent by the "11-oxysteroids." Because altered mineral metabolism appears to precede acute gouty attacks (2, 13), other studies with particular emphasis on "mineralo-corticoid" activity are now in progress at this laboratory.

During the preparation of this manuscript, the use of testosterone in gout was reported by Hollander and associates (40). In a small group of cases, testosterone combined with colchicine or ACTH appeared to offer therapeutic benefit. In our experience, testosterone alone in gout also seems to be effective. The use of this hormone deserves further trial in a larger number of cases of gout.

An inverse correlation between the levels of blood glutathione and blood sugar, as postulated by Conn *et al.* (33) to account for the diabetogenic effects of ACTH, was not found in this study. More recent work in this and other laboratories has similarly failed to demonstrate a constant relationship (41, 42).

SUMMARY AND CONCLUSION

1. Results of investigation of adrenal cortical function in a group of gouty patients during various stages of the disease, and the effects of ACTH, colchicine and testosterone are reported.

2. Significant change in adrenal activity was not found in the acute stage of the disease as contrasted with the interval phase. Some possible interpretations for this observation are discussed.

3. Colchicine, in therapeutic dosage, appeared to have no significant effect on the pituitary-adrenal systems of the patients studied.

4. The over-all response of these patients to exogenous ACTH was grossly within the accepted normal range.

5. The development of both the spontaneous and post-ACTH attacks of gouty arthritis did not seem directly related to the level of adrenal cortical activity.

6. Testosterone may have some therapeutic value in the treatment of acute gout. However, withdrawal of this steroid was found to precipitate gouty attacks in 5 of 9 patients.

7. A constant inverse relation between the blood sugar and glutathione during ACTH administration was not observed.

8. ACTH had a temporary beneficial action upon the subcutaneous tophi of one patient. Cortisone and testosterone had a similar but less marked effect.

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A METHOD FOR THE DETERMINATION OF 17-HYDROXYCORTICOSTEROIDS IN BLOOD: 17-HYDROXYCORTICOSTERONE IN THE PERIPHERAL CIRCULATION*

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ADRENAL steroid activity has been demonstrated in adrenal venous blood by means of biologic assay (1, 2). The biologic techniques, although successful in demonstrating the presence of adrenal steroids, have been unsatisfactory from a quantitative standpoint and have given little information regarding the specific compound being measured. Chemical methods for the determination of adrenal steroids in blood have been proposed by Corcoran and Page (3), and by Porter and Silber (4).

The method of Corcoran and Page was found to give high values due to the formation of formaldehyde from phospholipids, traces of which could not be eliminated even by repeated precipitation with acetone. The color reaction of Porter and Silber depends on the formation of phenylhydrazones in acid solution. Under the conditions given, the 17,21-dihydroxy-20-ketosteroids showed a strong maximum absorption at 410 mμ when several steroid ketones were tested. The purification procedure used with plasma, however, was so nonspecific as to raise the question of the influence of other compounds on the reaction.

The present communication deals with the development of a method for the quantitative estimation of 17-hydroxycorticosteroids in peripheral blood, using the color reaction described by Porter and Silber. The chief compound measured is apparently 17-hydroxycorticosterone[1] (5).

METHOD

Reagents:

1. Chloroform, U.S.P., redistilled from K_2CO_3 immediately before use.
2. Ether U.S.P., redistilled and washed with ferrous sulfate immediately before use.
3. Hexane (Skellysolve B), redistilled.

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[1] The 17-hydroxycorticosterone used in this study was kindly furnished by W. J. Haines of The Upjohn Company, Kalamazoo, Mich.

4. *a.* Magnesium silicate-Celite mixture: Magnesium silicate (No. 34, obtained from Philadelphia Quartz Company, Berkeley, California) and Celite analytic filter aid are mixed (1:1), washed with ethanol and dried. The mixture is then heated at 600° C. for four hours.

b. Florosil (60/100 mesh, obtained from Floridin Company, Warren, Pa.) is washed with ethanol, dried and heated at 600° C. for four hours.

5. Methanol, redistilled from 2,4,dinitrophenylhydrazine.

6. Dilute sulfuric acid (190 ml. H_2O:310 ml. conc. H_2SO_4).

7. Phenylhydrazine HCl, recrystallized from ethanol four times.

8. Phenylhydrazine-sulfuric acid solution (16 mg. of reagent 7 plus 10 ml. of reagent 6). This solution is prepared each day immediately before use.

Analytic procedure

Either 30-ml. samples of blood or 10-ml. samples of plasma have generally been used. Larger amounts can be employed but smaller amounts may lead to serious error if the values are low.

The blood is laked with an equal volume of water and extracted four times with a 4:1 ether-chloroform mixture (6). The extraction is carried out in 250-ml. centrifuge bottles so that the ether layer may be separated by centrifugation. Thorough mixture of the ether with the blood is obtained by vigorous agitation with glass rods. Shaking produces emulsions which are hard to break. The combined ether extracts are evaporated to dryness. The residue is then taken up in 20 ml. of 70 per cent ethanol and washed three times with 15 ml. of hexane in a small separatory funnel. The adrenal steroids remain in the ethanol fraction, and the hexane is discarded. The ethanol solution is taken to dryness in vacuum and the residue is dissolved in 5 ml. of chloroform for chromatography.

Plasma is extracted three times with 1.5 volumes of chloroform. The combined chloroform extracts are taken to dryness and dissolved in 5 ml. of chloroform for chromatography.

Chromatography on magnesium silicate-Celite columns: The chromatography is carried out on 1.5 Gm. of a 1:1 magnesium silicate-Celite mixture. The columns have an inside diameter of 11 mm. In the preparation of the column the magnesium silicate-Celite is mixed with 15 ml. of chloroform and poured onto a glass wool plug, after which 2–5 lbs. per sq. inch pressure of nitrogen is applied. More chloroform is added until the column is well packed. Another glass wool plug is then placed on top of the column and the solution of the sample in 5 ml. of chloroform is added. Elution from the column is carried out with 25 ml. of 2 per cent ethanol in chloroform, and 25 ml. of 15 per cent ethanol in chloroform. The 15 per cent ethanol contains the 17-hydroxycorticosteroids. This fraction is collected in two 12-ml. portions in test tubes and is evaporated to dryness with a stream of air. The sample is washed down to the bottom of each of these tubes and evaporated to dryness; the color reactions are run in the same tubes.

Chromatography on Florosil columns: A column of Florosil is prepared by tamping the adsorbent with a footed glass rod, as it is added to the 11-mm. column. A convenient length is 70 mm. of tightly packed material. The column is thoroughly wet with chloroform, and as the solvent drains to the upper level of the adsorbent the chloroform solution of the sample is added. The elution is carried out with 25 ml. of chloroform, 25 ml. of 2 per cent methanol in chloroform, and 45 ml. of 25 per cent methanol in chloroform. The last fraction contains the 17-hydroxycorticosteroids and is collected in a special 100-ml. boiling flask which has a small well blown in the bottom. The solution is evaporated to dryness in a 50° water bath with a stream of air. The sides of the flask are washed down

with a small amount of chloroform and again evaporated to dryness. The residue should now be entirely in the small well. The color reaction is run in the well, thus avoiding unnecessary transfers.

The sample is completely dissolved in 0.2 ml. of methanol, and 0.3 ml. of the phenylhydrazine-sulfuric acid solution is added. The tubes are shaken to give thorough mixing and then incubated at 60° C. for one hour. After incubation they are cooled in a cold water bath and placed in 0.5-ml. microcuvettes for reading on the Beckman spectrophotometer in the 370–450 mμ range. The maximum absorption at 410 mμ is measurable for quantities ranging from 0.5 to 10.0 gamma. Samples are read against a solvent blank run through the columns.

RESULTS

Figure 1 illustrates that the color which was produced followed Beer's Law when the reaction was carried out as described above. It also il-

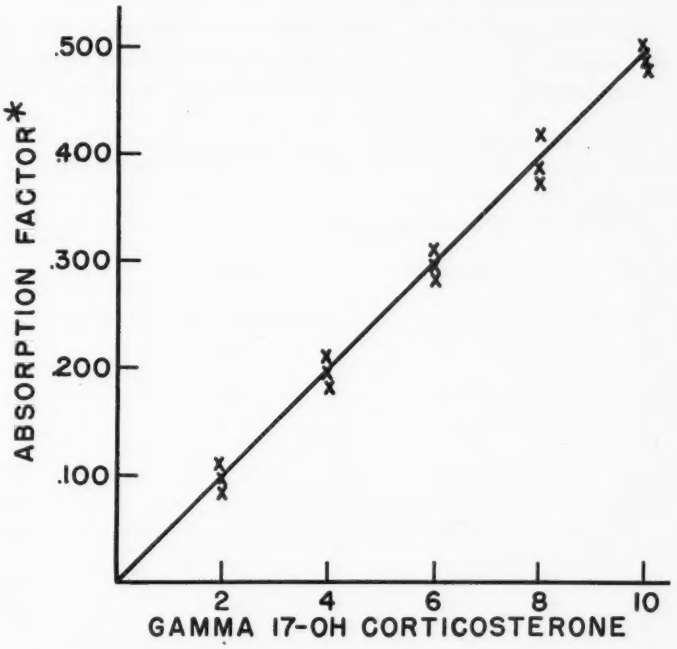

FIG. 1. Relation between absorption factor* and amounts of 17-hydroxycorticosterone.

* See text for explanation of term "absorption factor."

lustrates the sensitivity of the method. The transmittance is 75 per cent (absorbance 0.100) at the 1γ level, a level which can be measured with an accuracy of ±1 per cent as far as the instrument is concerned. The maxi-

mum range of variation with standards of this strength carried through the entire colorimetric procedure has been about 5 per cent.

When extracts of fresh blood are carried through the entire procedure there is a small amount of background absorption, which decreases with increasing wavelength. An approximate correction for this has been made by using the "absorption factor." This refers to a simple calculation of the increased absorption at 410 mμ compared with absorptions at 370 mμ and 450 mμ, as given in the formula:

$$\text{(absorption at 410 m}\mu\text{)} - \frac{\text{(absorption at 370 m}\mu + \text{absorption at 450 m}\mu\text{)}}{2} = \text{absorption factor (7).}$$

This assumes a uniform change in the background with wavelength, a condition which is probably not quite true.

Recoveries: Total recoveries of 17-hydroxycorticosterone added to blood, using the magnesium silicate-Celite column, are illustrated in Table 1. Table 2 demonstrates variations between duplicate samples of blood.

TABLE 1. RECOVERIES OF 17-HYDROXYCORTICOSTERONE EXTRACTED FROM 30 ML. OF WHOLE BLOOD WHEN MAGNESIUM SILICATE-CELITE COLUMN IS EMPLOYED

17-hydroxycorticosterone			Recovery of added steroid, %
Added, γ	Expected, γ	Found, γ	
2	4.3	4.0	85
2	4.3	4.4	105
2	4.3	4.4	105
3	4.6	4.3	90
3	4.6	4.1	83
3	4.6	4.4	93
4	4.8	4.9	102
4	4.8	4.8	100
4	4.8	5.3	112

Normal values for peripheral human blood appear to be from 4 to 10γ per 100 ml. of whole blood, as measured by this method. In 50 instances arterial and venous samples were drawn simultaneously. These included samples both before and after injection of ACTH. No consistent differences in the two levels were found. There was less than 1 per cent variation between the means of these groups.

TABLE 2. VALUES FOR 17-HYDROXYCORTICOSTERONE FOUND ON DUPLICATE SAMPLES OF THE SAME BLOOD CARRIED THROUGH THE ENTIRE ANALYTIC PROCEDURE

Sample	1		2		3		4		5	
	A	B	A	B	A	B	A	B	A	B
γ found	5.9	6.2	5.7	5.1	5.4	5.8	14.3	13.7	5.3	6.1

Recovery of known quantities of compound F from plasma, using the Florosil column, are shown in Table 3. Since losses with different batches of Florosil have been found to vary from 5 to 30 per cent, although variation between samples chromatographed at the same time is much less, it has been found advisable to run standards through columns alongside the unknowns. These standards are then used as the basis for calculation of values.

TABLE 3. RECOVERIES OF 17-HYDROXYCORTICOSTERONE EXTRACTED FROM 10 ML. OF PLASMA WHEN FLOROSIL COLUMN IS EMPLOYED

17-hydroxycorticosterone added, γ	Recovery of added steroid, %
1	95
2	85
3	95
4	92
1	112
2	116
3	92
4	91
1	82
2	132
3	83
4	103
1	55
2	107
3	91
4	98
1	80
2	105
3	102
4	115

Occasionally the Florosil columns give noticeable residues in the standard tubes. The reason for this is unknown. It has been found, however, that in such cases the background color is high and the calculated values show greater variation. It is advisable to discard such samples.

Evaporation of solutions under air jets: Known quantities of compound F have been evaporated to dryness with a stream of air under the conditions employed in the method, in order to rule out any destruction of the compound by air oxidation. Table 4 illustrates that such destruction does not occur.

TABLE 4. EFFECT OF AN EVAPORATION AND SOLVENT BACKGROUND ON ETHANOL-CHLOROFORM STANDARD SOLUTIONS

Steroid added, γ	Recovery, %
2	92
2	91
4	95
4	93
6	104
6	98

The compound in human peripheral blood which was measured by this reaction was identified by paper chromatography. The blood from a patient given ACTH was found to contain 45γ of 17-hydroxycorticosteroids per 100 ml. of blood. Another sample taken at this time was extracted in the manner described and then chromatographed on paper, using the method of Burton, Zaffaroni and Keutmann (8). The toluene-propylene glycol system was used and the chromatogram was allowed to develop for seventy-two hours. The sample was run simultaneously with known quantities of compound F and cortisone. The material obtained from blood moved at the same rate as compound F, and no evidence for the presence of cortisone was found.

DISCUSSION

The recoveries of 17-hydroxycorticosterone added to blood, together with the similar values found in duplicate samples from the same subject, indicate that the present method is adequate for this purpose.

Although the method described measures any 17-hydroxycorticosteroids which might be present in the blood, the evidence available (9, 10, 11) suggests that 17-hydroxycorticosterone is the chief compound secreted by

the adrenal cortex. The results of the paper chromatogram are in accord with this, and indicate that compound F is the chief 17-hydroxycorticosteroid in peripheral blood.

There are good reasons for the disagreement between the levels which are found by this method and those found by others. Paschkis (1) was able to demonstrate adrenal cortical activity in peripheral blood before administration of ACTH, but the data presented are not easily interpretable in terms of compound F. The production of formaldehyde by periodic acid oxidation, as used by Corcoran and Page (3), is dependent upon the purity of the steroids to be measured. Any primary or secondary hydroxyketone or any glycol group will yield formaldehyde. Thus traces of phospholipids will be hydrolyzed, the glycerol oxidized, and high values will result. The method of purification described by them does not completely eliminate such material and this may account for the extremely high values obtained. The color reaction used in the present method was previously applied to crude blood extracts, on a macro scale, by Porter and Silber. They were unable to identify any material in plasma after administration of cortisone. It seems unlikely, however, that the reaction applied in this manner would have measured the small quantities of 17-hydroxycorticosteroids which are now found to be present. The varying results of others are explicable, therefore, on the basis of poor isolation in the case of the high values, and insensitivity in the case of low or negative findings. The present method overcomes these factors by use of the chromatogram and the micro cell.

SUMMARY

A method has been described for the quantitative determination of adrenal steroids in peripheral blood. The method consists essentially of ether-chloroform extraction, partitioning of the ether-chloroform soluble material between 70 per cent ethanol and hexane, and chromatography of the ethanol-soluble fraction on a magnesium silicate-Celite or Florosil column. Quantitative determination of the 17-hydroxycorticosteroids is carried out by the use of a micro modification of the color reaction described by Porter and Silber.

Normal values for 17-hydroxycorticosteroids in human peripheral blood have been found to be between 4 and 10 gamma per 100 milliliters of whole blood.

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THE EXCRETION OF DEHYDROISOANDROSTERONE DURING ADRENAL STIMULATION WITH ADRENOCORTICOTROPIC HORMONE*

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DEHYDROISOANDROSTERONE is recognized as a steroid excretion product related to adrenal function, and it is excreted in large amounts by persons suffering from adrenal tumor (1, 2, 3) but not by those suffering from adrenal hyperplasia. These facts suggest that a study of this excretion product after stimulation of the adrenal cortex with adrenocorticotropic hormone (ACTH) would yield further information on the activity of the normal adrenal cortex. The recent contribution of Landau and associates (4), using the Pettenkofer reaction, again calls attention to dehydroisoandrosterone as an important excretion product connected with normal adrenal cortical activity.

The well recognized instability of dehydroisoandrosterone to the hydrolysis with strong acid at high temperature used by previous investigators, led to the use of milder hydrolytic procedures. As pointed out by Leiberman and Dobriner (5) mild acid hydrolysis appears to be sufficient to liberate the steroids conjugated as sulfate and certainly dehydroisoandrosterone sulfate. Their procedure of ether extraction at pH 1 for forty-eight hours at room temperature liberates between 75 and 90 per cent of the material (reacting to Allen's procedure for dehydroisoandrosterone) found in the crude butanol extracts of unhydrolyzed urine. The separation of the product extracted by ether at pH 1 into ketonic and nonketonic fractions, showed that 80 to 90 per cent was recovered in the ketonic fraction, with a barely measurable amount left in the nonketonic fraction. Equally good recovery of this material was found in the digitonin-precipitable fraction. The nature of the unhydrolyzed material which can be liberated by higher temperature and acid has not been carefully investigated but it contains only traces of steroid reacting to Allen's test.

METHODS

The method of Holtorff and Koch (6) was used for the determination of 17-ketosteroids. The total 17-ketosteroids were determined on separate

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* The generous supply of adrenocorticotropic hormone used in these experiments was furnished by Armour and Company through the courtesy of Dr. John Mote.

aliquots of ether-extracted acid-hydrolyzed urine. When crude extracts were used the color was extracted with chloroform, as recommended by Cahen and Salter (7).

The procedure of Allen, Hayward and Pinto (8) was used for the estimation of dehydroisoandrosterone and Allen's (9) formula was applied for the estimation of the quantity present when the absorption curves showed contamination by peaks at 390 and 480 mμ. As purification proceeded the absorption maximum at 480 mμ disappeared and that at 390 mμ was greatly reduced. Dehydroisoandrosterone was used as the reference standard in both determinations.

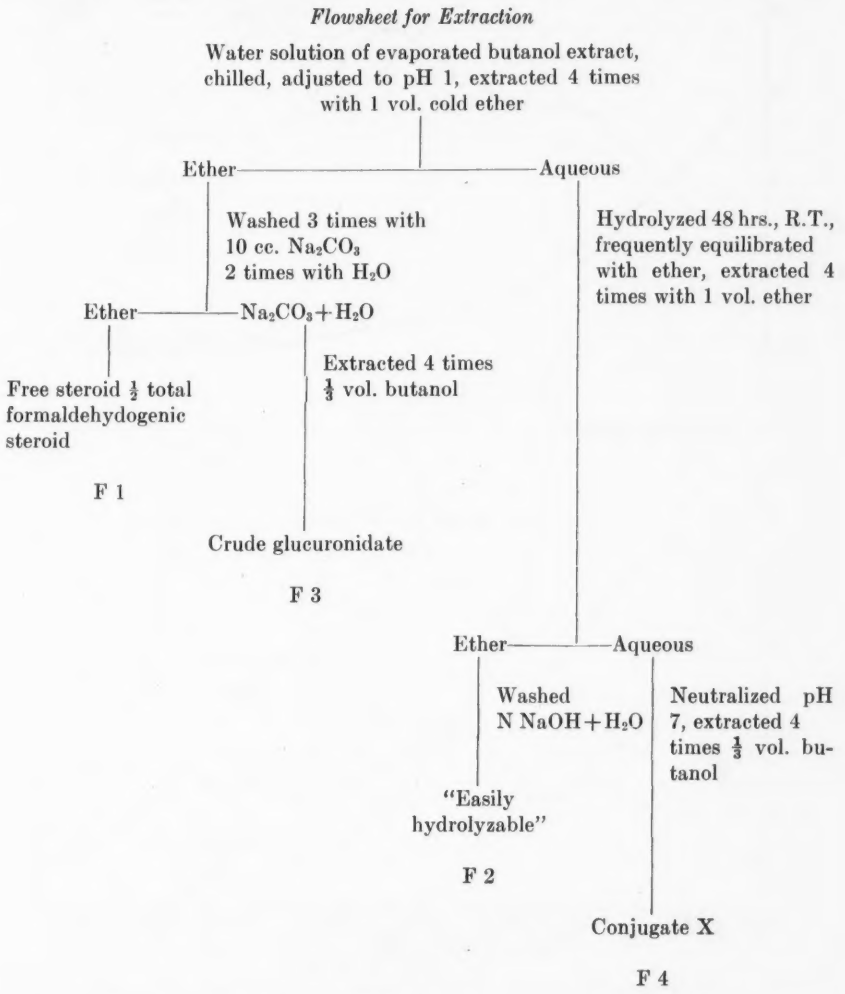
The extraction procedure used was that of Leiberman and Dobriner (5), with modifications indicated in the flowsheet. The starting material for separation of the conjugated steroids was an aqueous solution of the evaporated butanol extract of acidified urine. The extraction of the glucuronic acid conjugate with ether before hydrolysis at room temperature has the advantage of removing most of the brown pigments. After hydrolysis at pH 1 the ether extract was contaminated with indigo, indicating that the ethereal sulfates are also easily hydrolyzed. The recovery of the unhydrolyzed glucuronidate makes it possible to study the enzymatic cleavage products without resorting to hydrolysis with strong acid. The nature of the residual conjugate has not been elucidated, it was therefore decided to recover this in unhydrolyzed form. The analysis of these two conjugates will be the subject of a later report.

EXPERIMENTAL

On the four crude fractions thus separated, 17-ketosteroids and the "blue" color of Allen's reaction for dehydroisoandrosterone were estimated. Fraction 1 (free steroid) gave an atypical color with meta-dinitrobenzene and no maximum at 600 mμ with Allen's reagent, but contained about half of the total formaldehydogenic material that could be extracted after 48-hour hydrolysis at room temperature. Fraction 2 (easily hydrolyzable fraction) contained 30 to 40 per cent of the total 17-ketosteroids, and 95 to 90 per cent of material reacting as dehydroisoandrosterone. Fraction 3 (the glucuronidate conjugate), although it contained only small amounts of material which gave a "blue" reaction with Allen's reagent, did contain considerable amounts of 17-ketosteroid. The nature of the blue-reacting material has not been determined. Fraction 4 (X conjugate) contained from one-quarter to one-third of the total 17-ketosteroids, but only a trace of material reacting as dehydroisoandrosterone. Since the sum of the 17-ketosteroids of the three fractions (omitting Fraction 1) was within 10 per cent of the total ketosteroids determined in the usual manner on an aliquot

of the original urine, this extraction procedure appears to give satisfactory recovery of steroids present.

Since Fraction 2 contained nearly all of the material reacting as de-


Flowsheet for Extraction
Water solution of evaporated butanol extract, chilled, adjusted to pH 1, extracted 4 times with 1 vol. cold ether
Ether
Aqueous
Washed 3 times with 10 cc. Na_2CO_3 2 times with H_2O
Hydrolyzed 48 hrs., R.T., frequently equilibrated with ether, extracted 4 times with 1 vol. ether
Ether
$Na_2CO_3 + H_2O$
Extracted 4 times $\frac{1}{3}$ vol. butanol
Free steroid $\frac{1}{2}$ total formaldehydogenic steroid
F 1
Crude glucuronidate
F 3
Ether
Aqueous
Washed N NaOH + H_2O
Neutralized pH 7, extracted 4 times $\frac{1}{3}$ vol. butanol
"Easily hydrolyzable"
F 2
Conjugate X
F 4

hydroisoandrosterone, it was this fraction that was used for further identification. After daily determinations of 17-ketosteroids and dehydroisoandrosterone in Fraction 2, the remaining extracts were pooled for each period as follows: 1) preliminary control period, 2) ACTH period, and 3) re-

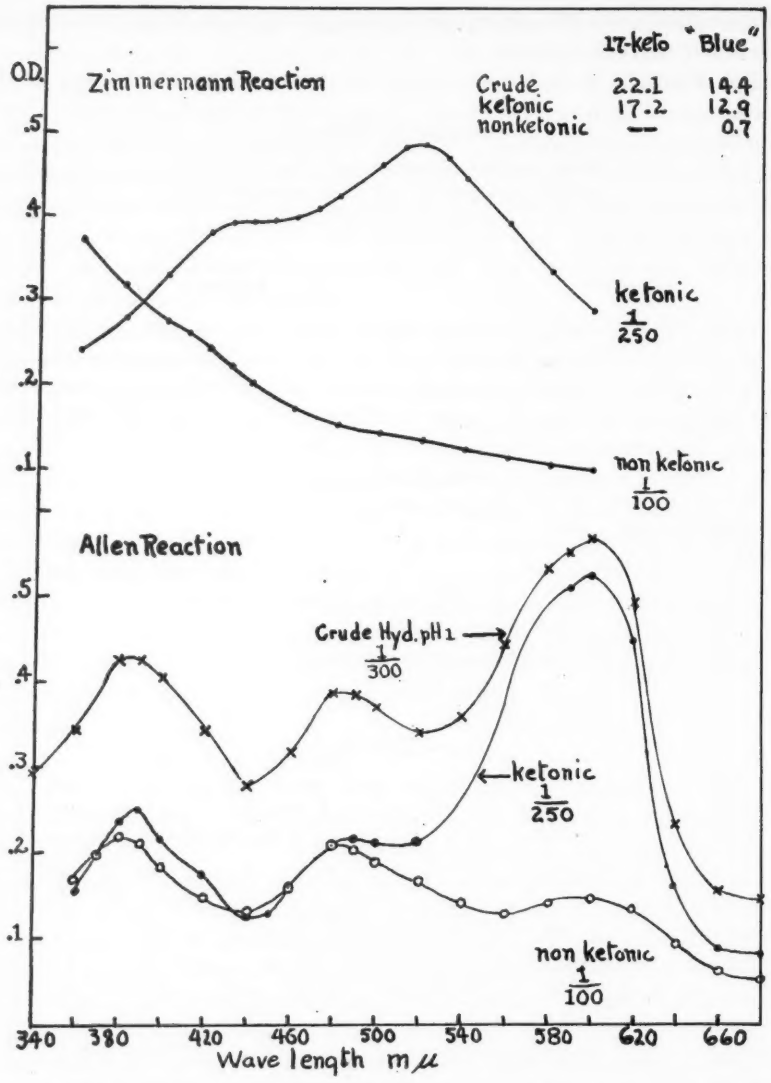


FIG. 1. The absorption spectra of the Zimmermann and Allen color products of the ketonic fraction of the easily hydrolyzable steroids from urine collected during ACTH administration. Patient A.T.

covery period. On samples from these periods, ketonic and nonketonic constituents were separated by Girard's reagent T, using the conditions recommended by Dobriner, Leiberman and Rhoads (11). The alcoholic and

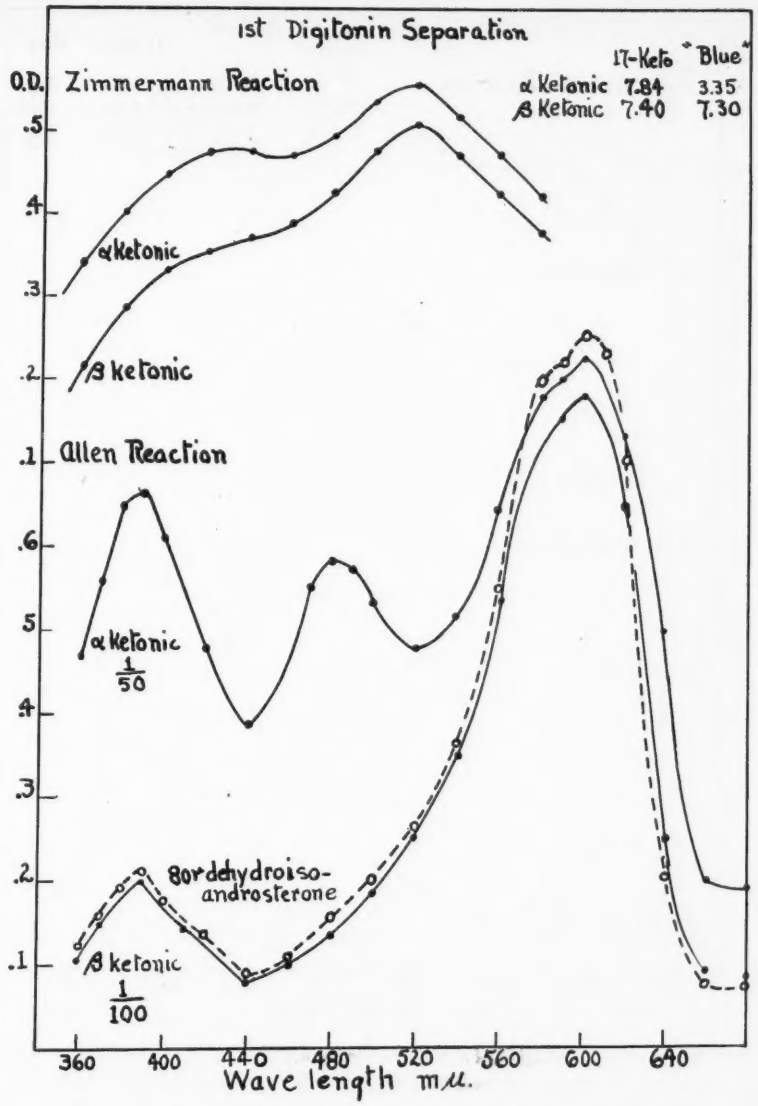


FIG. 2. Absorption spectra of the products of the first digitonin separation of the ketonic fraction.

ketonic fractions were both tested for the presence of substances giving rise to the "blue" color when treated by Allen's procedure. There was but a trace of reacting material found in the nonketonic fraction.

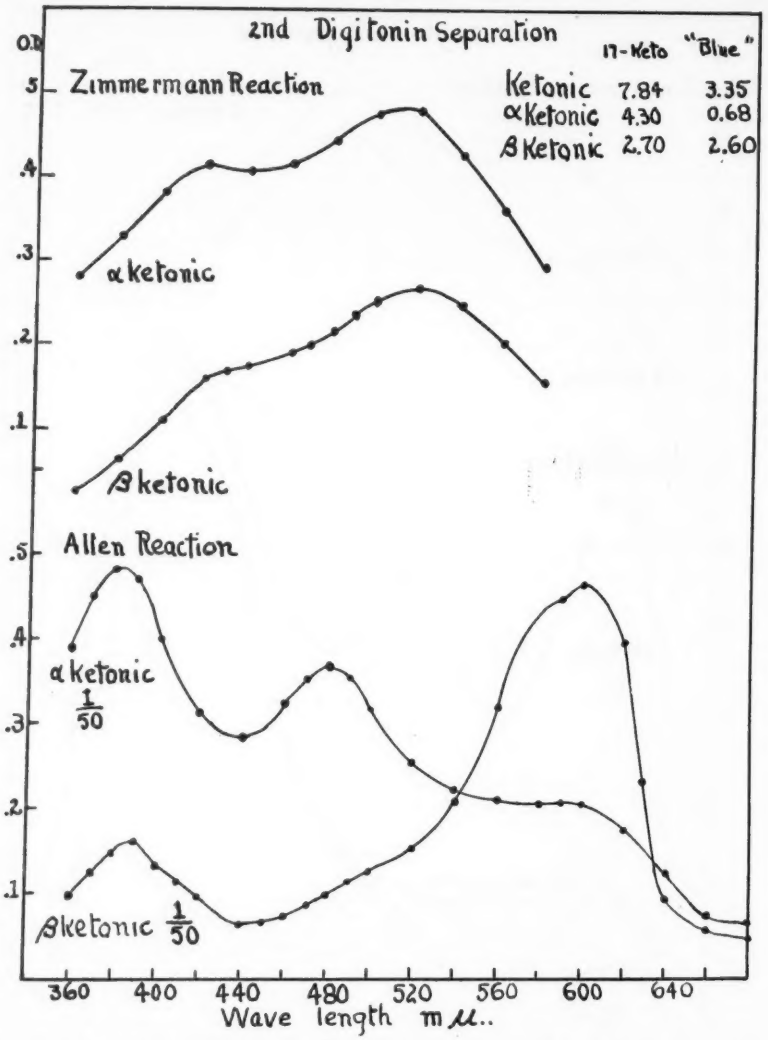


FIG. 3. Absorption spectra of second digitonin separation of the ketonic fraction from first separation, showing nearly all of the 600 mμ absorption of Allen's "blue" color to be in the ketonic fraction.

Keeping in mind the limitations of the digitonin separation of α and β steroids, the conditions outlined by Dobriner *et al.* (11) were adhered to. With the exception of one sample, all but a trace of the "blue" reacting compound was precipitated. On this sample the α fraction was reprecipi-

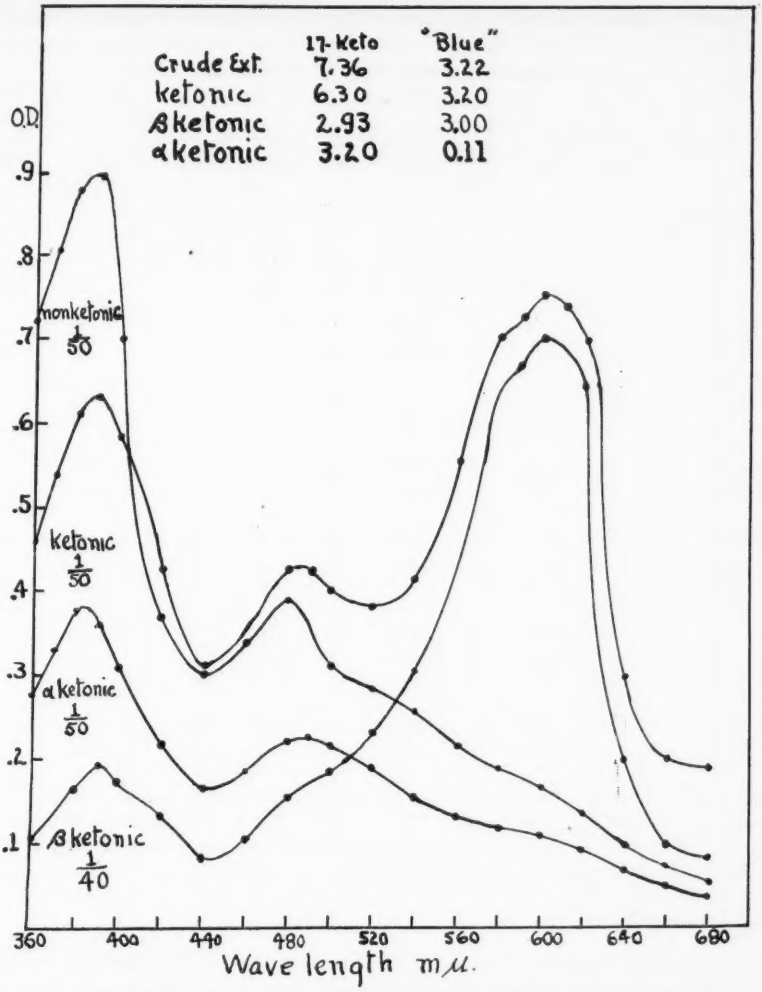


FIG. 4. Absorption spectra of Allen's "blue" color after a single digitonin separation of ketonic fraction, from the control period of patient A.T., showing that separation of the α and β components may be essentially complete.

tated with digitonin, with the result that the blue color was then found in the β fraction. The changes in the absorption curves of the materials separated are shown in Figures 1, 2, 3. The decrease in the absorption at 600 mμ in the pigment given by the α fraction and the presence of this absorption in the pigment given by the β fraction clearly indicate that this method of extraction causes no change in the dehydroisoandrosterone.

TABLE 1. ALPHA AND BETA FRACTIONATION OF EASILY HYDROLYZABLE STEROID

Patient	Sex	Total ketonic			Alpha ketonic		Beta ketonic		Recovery of alpha+beta ketonic steroids		Non-ketonic "blue" (mg.)
		17-KS (mg.)	"Blue" (mg.)	"Blue" / 17-KS (%)	17-KS (mg.)	"Blue" (mg.)	17-KS (mg.)	"Blue" (mg.)	(mg.)	(%)	
A.T. 1*	F	6.3	3.2	51	3.1	.11	2.8	2.9	5.9	93	.02
A.T. 2**		17.2	12.0	70	4.3	.68	10.1	10.0	14.4	84	.70
A.T. 3†		3.3	.5	13	2.6	.00	.4	.5	3.0	86	.00
D.V. 1	F	5.6	2.3	44	3.0	.01	2.2	2.3	5.3	94	.08
D.V. 2		9.6	4.7	49	4.4	.03	4.5	4.4	8.9	93	.05
G.S. 1	F	5.8	1.8	31	3.6	.03	1.7	1.7	5.3	91	.08
G.S. 2		5.1	1.5	30	3.2	.01	1.3	1.2	4.5	88	.06
M.S. 1	F	4.6	1.5	32	2.9	.00	1.4	1.4	4.3	93	—
M.S. 2		8.9	3.6	42	4.8	.11	3.4	3.4	8.2	92	—
G.D. 1	F	9.4	3.5	37	5.2	.12	3.3	3.2	8.5	90	.10
G.D. 2		12.4	6.1	49	5.8	.11	5.6	5.8	11.4	92	.31
G.D. 3		3.4	.4	14	2.6	—	0.3	0.3	2.9	85	—
A.E. 1	M	5.2	2.1	41	2.6	.03	1.9	1.9	4.5	86	.03
A.E. 2		7.9	4.0	51	3.6	.05	3.5	3.8	7.1	89	.01
A.E. 3		4.1	.5	12	3.1	—	0.4	0.4	3.5	86	.00
E.W. 1	M	7.4	3.5	47	3.4	.10	3.3	3.4	6.7	90	—
E.W. 2		8.9	4.5	51	3.8	.09	4.1	4.2	7.9	90	—
Known 1		10.0	5.0	50	4.5	.00	4.7	4.7	9.2	92	—
Known 2		10.0	2.0	20	7.7	.00	1.8	1.8	9.5	95	—

* 1 = preliminary period.
** 2 = ACTH period.
† 3 = recovery period.

Figure 2 also shows that the absorption curve of the purified β-ketonic fraction reacts to Allen's procedure in a manner identical to that of authentic dehydroisoandrosterone. Moreover, as pointed out by Dobriner *et al.* (11), only β-ketones appear in the digitonin precipitate, but their precipitation is not always complete. The reaction with meta-dinitrobenzene is also typical, leaving little doubt that a 17-ketosteroid of the β configuration (dehydroisoandrosterone) is the substance responsible for the "blue" color. The close correspondence between the values observed by the two procedures indicates that in the β-ketonic fraction there are only traces of other steroids, such as have been isolated from hot acid hydrolysates of urine by Leiberman *et al.* (5). The absorption curve of the Zimmermann

TABLE 2. DEHYDROISOANDROSTERONE (BLUE) AND 17-KETOSTEROID EXCRETION

Subjects	Sex	Total 17-KS (mg./24 hrs.)	Hydrolyzed pH 1 "blue" (mg./24 hrs.)	"Blue" / 17-KS %
Normal				
H	M	23.0	5.0	21.7
S	M	16.8	3.7	22.0
F	M	14.8	2.4	16.2
G	M	13.8	2.1	15.2
R	F	7.6	2.0	26.0
H	F	12.2	2.6	21.0
S	F	10.6	2.5	24.0
Patients				
EW 1*	M	10.8	2.0	18.9
EW 2**		40.6	9.3	22.9
EW 3†		6.2	.6	19.7
MS 1	F	12.0	2.0	16.7
MS 2		52.0	10.2	19.6
MS 3		10.2	1.0	9.8
DV 1	F	13.6	2.1	16.0
DV 2		34.8	6.2	18.2
DV 3		6.3	.5	7.9
DV 2a‡		59.0	9.4	16.0

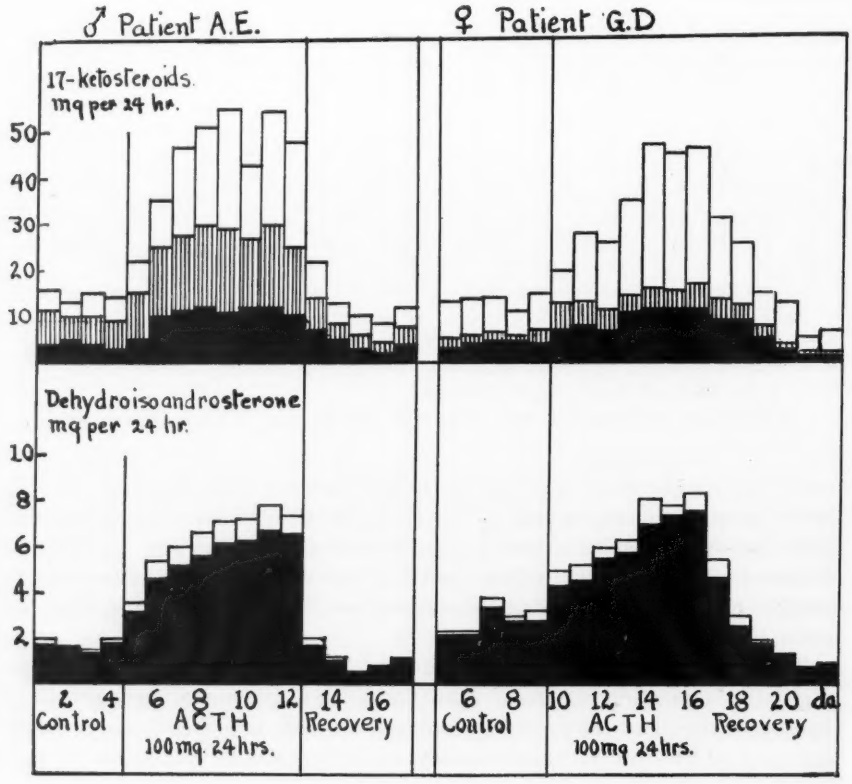
Values for normal subjects represent averages of analyses of 3 collections.
Values for patients:
* 1 = control period—average of 2 days.
** 2 = fourth and fifth days of ACTH—100 mg. per 24 hours in four doses.
† 3 = third and fourth days of recovery.
‡ 2a = tenth and eleventh days of ACTH—150 mg. per 24 hours in four doses.

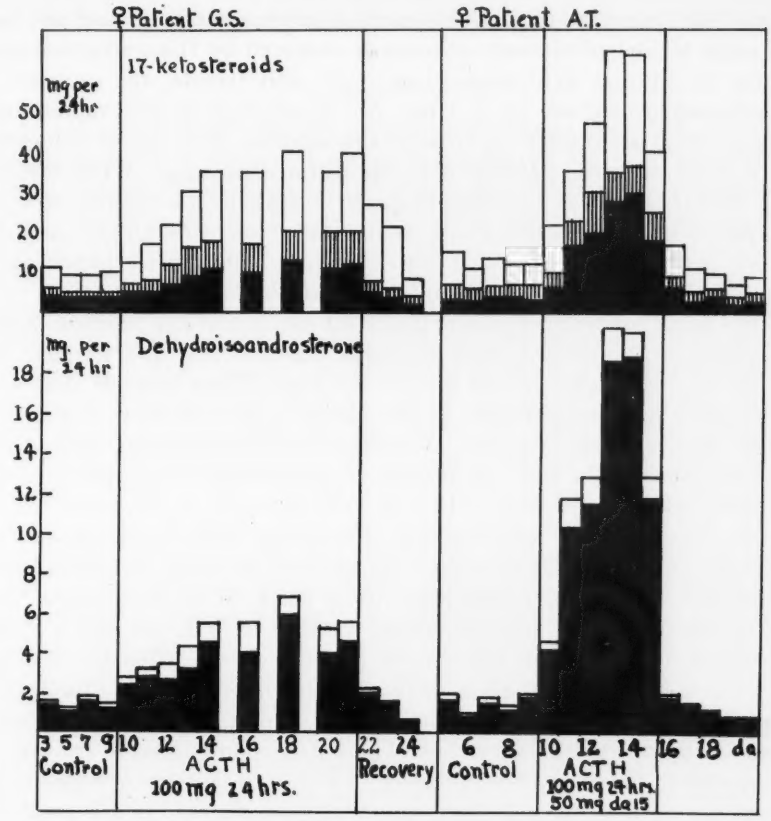
color on the α-ketonic fraction was in most cases typical of that of the 17-ketosteroid standard, though in two cases there could have been considerable contamination as shown by an accentuated absorption at 420 mμ. Figure 4 indicates that the first digitonin precipitation may be essentially complete. When the α fraction contained no "blue" reacting material the second precipitation was omitted.

Table 1 shows the values for α and β ketonic fractions, as found after digitonin separation in the samples used. It can be seen that the total ketonic fraction of the easily hydrolyzed steroids contains from 31 to 68 per cent of the total 17-ketosteroids as dehydroisoandrosterone ("blue"

color with Allen's procedure), both in the initial period and during ACTH administration. In four of the six experiments there was a significant increase in the relative amount of β-ketosteroid. However, in the recovery period, in the 3 patients examined, it dropped to 12 or 13 per cent of the total ketosteroids of this fraction.

Only 70 to 80 per cent of the steroids estimated in the crude fraction by the Zimmermann reaction could be recovered in the ketonic fraction. Analysis of absorption curves showed this to be due to overestimation in the crude extracts. The ketonic fractions were considerably more reliable and the recovery of 17-ketosteroids in the α and β fractions was from 82 to 93 per cent of the total ketonic fraction. Girard's and digitonin separation of a known mixture of androsterone, dehydroisoandrosterone and pregnanediol showed from 92 to 95 per cent recovery. The total ketonic fraction still apparently contained some impurities, adding to the Zimmermann color.





FIGS. 5 and 6. 17-Ketosteroid and dehydroisoandrosterone excretion in patients treated with ACTH, 100 mg. in four doses per 24 hours. The upper chart in each figure shows 17-ketosteroid (black area = "easily hydrolyzable"; shaded area = glucuronidate). The lower chart in each figure shows dehydroisoandrosterone (black area = "easily hydrolyzable"; clear area = glucuronidate).

The β-ketonic fraction gave typical absorption spectra in both the Zimmermann reaction and Allen's reaction for dehydroisoandrosterone. The amount of the total "blue" color recovered in the β-ketonic fraction was from 85 to 95 per cent of that estimated in the total ketonic fraction. Only traces of "blue" reacting material remained in the α-ketonic fraction. These traces may have been due to β-ketones that had escaped digitonin precipitation.

Since 85 to 90 per cent of steroid reacting as dehydroisoandrosterone is found in the "easily hydrolyzable" fraction as β-ketosteroid, Allen's procedure can be used with considerable confidence on this fraction. Table 2

shows daily values of total 17-ketosteroids after hot acid hydrolysis, and the value for dehydroisoandrosterone as obtained by this extraction procedure. In normal individuals, both male and female, the amount of dehydroisoandrosterone (2 to 5 mg. per twenty-four hours), represented from 15 to 26 per cent of the total 17-ketosteroids. The control values for the 3 psychoneurotic patients also fell within this range. When treated with ACTH there was a marked absolute increase in this fraction to 6–10 mg. per twenty-four hours, and a small relative increase. In the early recovery period the values were lower than in the initial period, and amounts relative to the 17-ketosteroids, were also reduced.

Since these preliminary observations showed a marked increase in dehydroisoandrosterone the daily excretion was followed in 4 patients receiving ACTH. Figures 5 and 6 show the total 17-ketosteroids (hot acid hydrolysis), and the amounts in the glucuronidate fraction and in the "easily hydrolyzable" fraction. Although the Zimmermann reaction applied to either of the two fractions gives a satisfactory absorption curve, the total value is overestimated by 10 to 15 per cent. In the lower half of Figures 5 and 6 are plotted the dehydroisoandrosterone value in the two fractions. During ACTH treatment, all patients showed a marked increase in excretion of total 17-ketosteroid. About 30 to 40 per cent of the total 17-ketosteroid is easily hydrolyzed and another 15 to 30 per cent is in the glucuronidate fraction in the control periods. This relationship changes but little as the 17-ketosteroids increase with adrenal cortical stimulation. The "blue" color is mainly in the easily hydrolyzable fraction. In all 4 cases, during the preliminary control period, this amounted to from 13 to 20 per cent of the total 17-ketosteroid. In 3 of the 4 patients in whom daily excretions were estimated, there was a marked increase in the total 17-ketosteroid and a proportional increase in the dehydroisoandrosterone, the ratio of the two remaining approximately constant. In the fourth patient (A.T.) the increase in total 17-ketosteroid was of the same order as that usually noted, but the increase in dehydroisoandrosterone was much greater and rose from 14 per cent to around 30 per cent of the total 17-ketosteroid (from 2–3 mg. per day to 20–22 mg. per day).

The glucuronidate fraction contained but traces of "blue" reacting material during the control and recovery periods, but on stimulation of the adrenal cortex with ACTH, from 1 to 1.5 mg. appeared in this fraction and maximally amounted to 20 per cent of the dehydroisoandrosterone determined.

The psychiatric and some of the metabolic responses of the patients to ACTH have been reported (Gildea, Ronzoni and Trufant (12)), and the only respect in which patient A.T. differed markedly from the others was

that she became so disturbed on the sixth day of treatment that ACTH was discontinued.

DISCUSSION

The values for dehydroisoandrosterone corroborate the recent report of Landau *et al.* (10) that this substance is associated with adrenal cortical activity; but no values were as high as the 48 to 63 per cent of the total ketonic fraction which they reported. The control values in psychoneurotic patients of 13 to 20 per cent of the total 17-ketosteroids as dehydroisoandrosterone, are not significantly lower than the 15 to 26 per cent observed in normal subjects and are similar to those found by Bitman and Cohen (13). At least it can be claimed for this procedure that the material determined in the "easily hydrolyzable" fraction is dehydroisoandrosterone, because of the identical absorption spectra of the "blue" color of Allen's reactions, and because of its appearance in the β-ketonic fraction. The presence of but traces of "blue" color in the more difficultly hydrolyzable fraction led to the conclusion that extraction of dehydroisoandrosterone had been substantially complete. The identity of the "blue" reacting material in the glucuronidate fraction remains to be determined.

The determination of dehydroisoandrosterone by this procedure and the considerable increases during adrenal cortical stimulation by ACTH suggest that excretion of this substance is as important an index to adrenal cortical activity as "corticoid" excretion. The procedure outlined is simple and the close correspondence between the total "blue" color from the ketonic steroids and from the isolated β-steroid indicate that substantially all of the steroid reacting in Allen's procedure is dehydroisoandrosterone.

The differences in the responses of individual patients are noteworthy, but so far we have been unable to correlate this difference with any known metabolic activity occurring on stimulation of the adrenal cortex with ACTH.

SUMMARY

1. The method of extraction at pH 1 yields 75 to 90 per cent of those steroids which react as dehydroisoandrosterone with Allen's procedure.

2. From 90 to 95 per cent of this material is recovered as β-ketones and accounts for from 30 to 70 per cent of the total 17-ketosteroids in this fraction.

3. The quantity of dehydroisoandrosterone normally excreted varies from 2.2 to 5.0 mg. per twenty-four hours and amounts to between 15 and 26 per cent of the total 17-ketosteroids in normal subjects.

4. By stimulation of the adrenal gland with ACTH, the amount of dehydroisoandrosterone excreted is increased to values ranging from 8 mg.

to as much as 22 mg. in twenty-four hours and may amount to 30 per cent of the total 17-ketosteroids.

Acknowledgment

The helpful advice and encouragement of Dr. Willard Allen are gratefully acknowledged. Dr. Trufant and Dr. Greditzer were also helpful in caring for the patients on the wards, and we are very grateful for the cooperation of the nursing staff who made adequate urine collections possible. This work was done with the technical assistance of Joan Boniface and Harvey Walker, Jr.

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THE EFFECT OF MASSIVE CORTISONE THERAPY ON MEASUREMENTS OF THYROID FUNCTION*

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THE employment of extremely large doses of cortisone acetate in the therapy of various disease states has recently provided a unique opportunity to explore the magnitude of changes in the functional state of the thyroid gland which may be induced by high levels of circulating 11-dehydro-17-hydroxycorticosterone. A decreased collection of radioiodine in the thyroid gland of the rat given cortisone has been demonstrated (1), and a decrease in the I^{131} "accumulation gradient" induced by moderate doses of cortisone (100 mg. daily) has been reported in man (2, 3). These same authors and others (4) have reported a decrease in blood protein-bound iodine levels incident to cortisone therapy in moderate doses.

It was the purpose of this study to observe the development, extent and duration of the suppression of radioiodine uptake, the changes in PBI, and, to a lesser extent, the changes in serum cholesterol which might be produced by massive cortisone administration in man.

METHODS

Eleven patients with normal thyroid function and 2 with exophthalmic goiter were included in this study. All patients received cortisone acetate, either orally or by intramuscular injection. Dosage ranged from 400 to 500 mg. per day, with the exception of one 5-year-old male who received 300 mg. per day, over periods ranging from several days to three weeks. The cortisone was given in the treatment of conditions including rheumatoid arthritis, rheumatic fever, the nephrotic syndrome and exfoliative

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dermatitis. During the period of therapy incidental observations on thyroid function were made.

Studies with tracer doses of I^{131} were carried out in 9 of the subjects before and after treatment. The tracer was repeated more than once in 3 of the subjects. Tracers were prepared by adding from 30 to 50 microcuries of carrier-free I^{131} to 25 cc. of tap water in a 50-cc. beaker or in 10-cc. vials containing sterile saline solution for intravenous injection. Solutions were counted with a heavily shielded gamma counter (Sylvania GG306), mounted so that the effective counting center of the tube was 46 cm. from a fixed point at which solutions were counted, and the activity over the thyroid region determined at intervals during the twenty-four hours subsequent to the administration of I^{131}. Activity over the thigh was subtracted as background, and the uptake in the gland at any given time was expressed as a percentage of the administered tracer activity, without backscatter correction and without additional geometric correction, except in the case of the small flask used for intravenous tracers. The rate of uptake by the gland during the first three to four hours was also expressed as the "accumulation gradient" (5) by dividing the percentage uptake by the square root of the time in minutes after administration. Urinary excretion of I^{131} was determined in 6-hour fractions over the 24-hour period, employing a dip counter.

Blood samples were drawn repeatedly before, during, and after treatment, for determination of the protein-bound iodine (PBI) by the method of Barker (6).

Cholesterol determinations were made by a titrimetric digitonin method employing hemolysis as an end point, as developed by White and Mann (7).

EXPERIMENTAL DATA

Control studies

Measurement of the thyroidal accumulation in twenty-four hours is the method now in use in this hospital for diagnostic studies with tracer doses of I^{131}. To ascertain the reproducibility of the 24-hour uptake, an analysis was made of results obtained in the 10 patients who had a repeat tracer of I^{131} without intervening treatment. Five of these had been re-tested within twenty-four to forty-eight hours, and the remainder had had tracers repeated after a period up to two months in duration. Expressing the percentage of the administered dose accumulated in twenty-four hours as an absolute figure, the range of variation between the repeated determinations was 0–4 (Table 1), and the mean variation was 1.9, with a standard deviation of 1.4. Stanley and Astwood have previously shown the reproducibility of the "accumulation gradient" of closely repeated tracer doses of I^{131} (8).

Because it is not certain that the "accumulation gradient" (representing the early rate of iodine collection by the gland) and the 24-hour accumulation necessarily reflect the same function, both expressions have been included in this study. The relationship between the gradient and the 24-hour uptake was found to be in agreement with that reported by Greer (9).

The relative constancy of the PBI in healthy adults has been previously reported by Danowski *et al.* (10). They found the maximum spontaneous variation in a euthyroid subject over a 4-week to 7-week period to be 1.1 micrograms per cent.

TABLE 1. REPRODUCIBILITY OF THE 24-HOUR PERCENTAGE ACCUMULATION OF I^{131} BY THE THYROID GLAND

Patient	1st uptake (per cent)	Interval	2nd uptake (per cent)
A	37	48 hours	38
B	57	48 hours	58
C	43	48 hours	44
D	42	48 hours	38
E	21	48 hours	21
F	62	7 days	60
G	39	9 days	36
H	92	30 days	93
I	56	60 days	52
J	38	60 days	36

Thyroidal depression in euthyroid individuals

Without exception, all 7 of the euthyroid patients studied with a repeated tracer of I^{131} demonstrated a marked decrease in accumulation of radioiodine in the thyroid region after the administration of cortisone (Table 2). This was reflected in proportional decreases in both the 24-hour percentage accumulation and the "accumulation gradient." The 24-hour accumulation in 2 of the subjects represented deductions from the total 24-hour urine excretion. There was an average difference of 17.2 (expressed in absolute numbers) between the 24-hour control uptake and the 24-hour uptake during therapy, as compared with the normal variation of 1.9 in repeated uptakes. The values obtained during treatment represented an average depression of radioiodine collection in twenty-four hours of approximately 50 per cent. The extent of depression was independent of the route of cortisone administration employed.

As a corollary to measurement of thyroidal accumulation, urinary excretion of the tracer was studied in 2 of the patients (A.S. and E.M., Fig. 1). Measurements were made in 6-hour urinary fractions over the 24-

TABLE 2. DEPRESSION OF THE RADIOIODINE UPTAKE OF THE THYROID GLAND IN EUTHYROID PATIENTS BY MASSIVE CORTISONE DOSAGES

Patient	Cortisone treatment		24-Hour uptake of I^{131} (%)	
	Duration (days)	Total (mg.)	Before cortisone	After cortisone
A.C.	4	2,000	29 (0.8)*	12 (0.2)
L.P.	4	2,000	21 (0.8)	3 (0.1)
R.G.	5	2,500	64 (3.7)	49 (1.4)
B.R.	14	7,000	47 (1.3)	29 (0.9)
A.S.	14	4,200	27	9
L.T.	16	5,300	39	26
J.L.	4	1,600	39 (1.2)	17 (0.5)

* Figures in parentheses represent accumulation gradients.

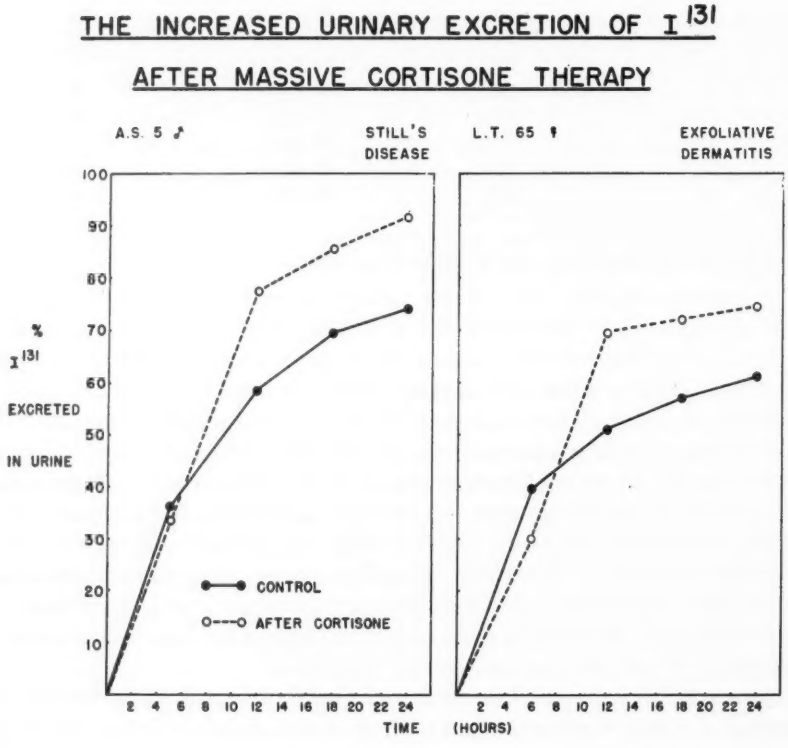

FIGURE 1

hour period. After cortisone, a significant increase in excretion of the tracer was observed in both patients.

To eliminate the possibility of an altered gastric absorption of I^{131} during cortisone administration, 1 patient (L.P., described later) was studied with intravenously administered tracers throughout the period of observation. The same depression was observed as with the oral tracers.

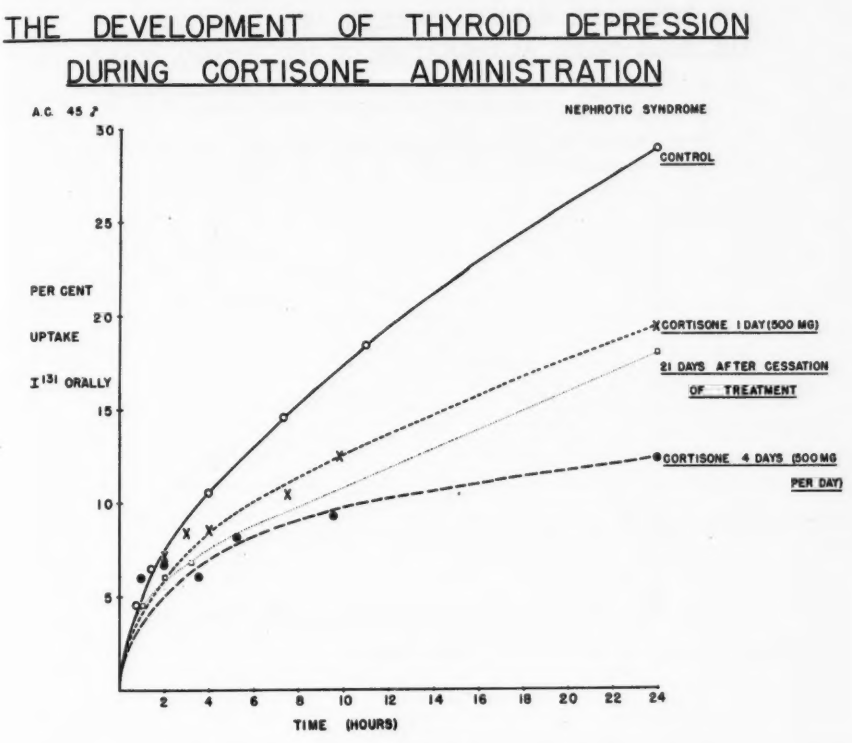

FIGURE 2

Temporal aspects of depression of uptake

Depression of the radioiodine accumulation in the thyroid gland after massive doses of cortisone may occur rapidly and persist for variable periods of time, depending upon the route of administration of the steroid. The depth of depression in radioiodine accumulation as measured by this method did not appear to depend upon the duration of treatment. The patient shown in Figure 2 received 500 mg. of cortisone intramuscularly for five days. Previous to treatment his uptake of I^{131} was 28 per cent in

twenty-four hours (accumulation gradient, 0.8). Following one day of treatment his collection of the tracer in twenty-four hours was significantly reduced to 19 per cent (gradient, 0.5), whereas after four days of cortisone there was further reduction to 12 per cent (gradient, 0.2). Twenty-one days after the cessation of cortisone the uptake of radioiodine was still significantly depressed, the twenty-four hour value at this time being 18

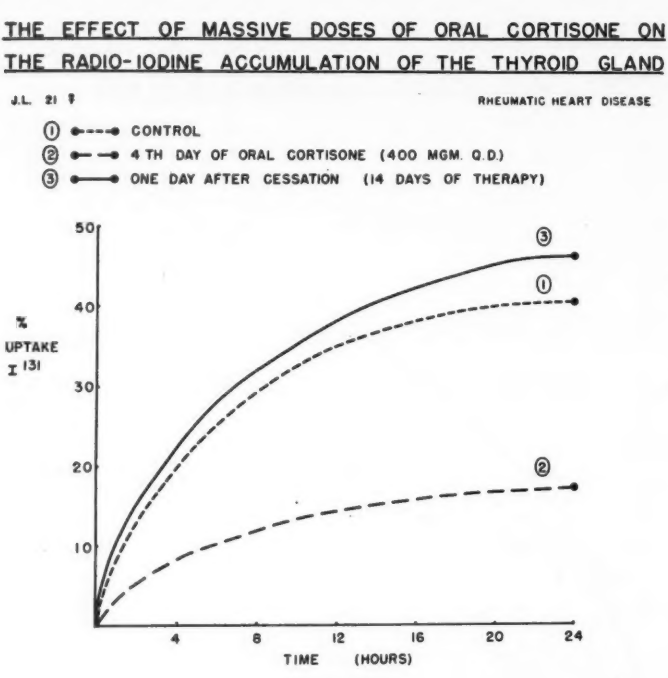

FIGURE 3

per cent (gradient, 0.4). This prolonged persistence of effect was not observed, however, in the case of massive doses of cortisone administered orally (Fig. 3). These findings are consistent with the prolonged "depot" effect of intramuscular cortisone when compared with treatment by the oral route, as emphasized by Thorn *et al.* (11).

The effect of thyrotropin (TSH)

The effect of administering thyrotropic hormone during the phase of depressed uptake is shown in Figure 4. This patient (L.P.) received continuous treatment for three weeks and demonstrated the most profound level of depression. After four days of treatment his control values of 21

per cent accumulation in twenty-four hours (gradient, 0.8) were reduced to the range usually seen in severe hypothyroidism, an uptake of 3 per cent in twenty-four hours (gradient, 0.1). On the fifth day of treatment thyrotropin was administered intramuscularly in four 12.5-mg. doses

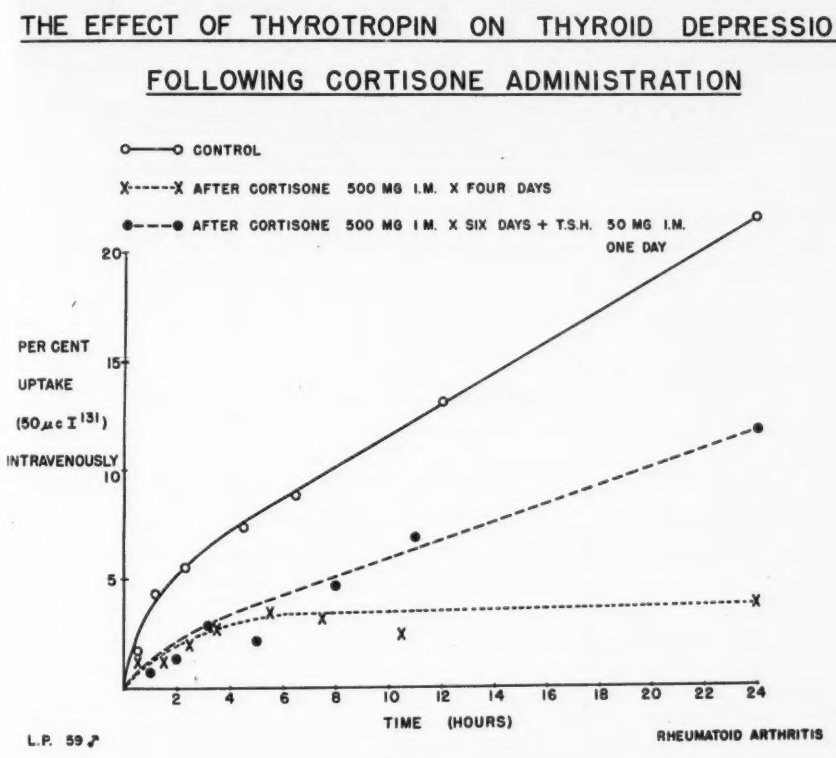

FIGURE 4

during the twenty-four hours, and on the following day his uptake had risen to 10 per cent (gradient, 0.3).

This admittedly single observation shows a significant difference and confirms a previous finding of Hill *et al.* (2). It appears to demonstrate that exogenous thyrotropic stimulation can increase radioiodine accumulation even when such accumulation has been markedly altered during cortisone administration.

Changes in serum protein-bound iodine (PBI) and cholesterol

Repeated protein-bound iodine determinations were made in 7 patients in the series. Changes are presented in Figure 5, in which the values ob-

tained during the period of treatment and two weeks after the cessation of cortisone are given as the percentage of the control PBI level. All patients showed a fall in the PBI concentration after cortisone treatment, the range of the decrease being from 12 to 60 per cent of the control value. Assuming approximately a 25 per cent decrease to be significant (10), 5 of the 6 patients whose PBI levels were followed after cessation of therapy showed a decrease of at least this magnitude. In several patients

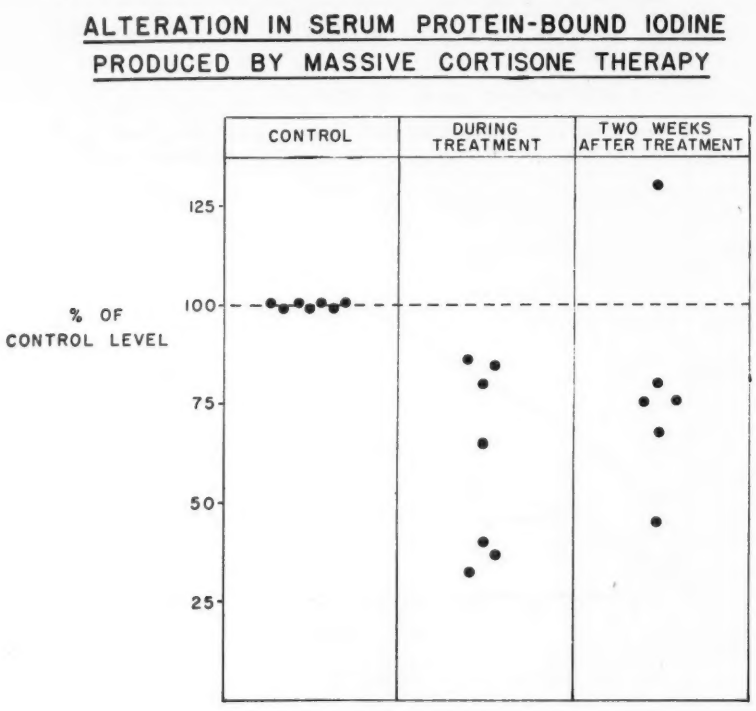

FIGURE 5

the maximum decrease was obtained during the actual period of therapy; in others, the maximal fall occurred later, in the first several weeks following the end of the treatment period, with an upward trend at the end of this time. The maximal fall was found in the patient (L.P.) whose PBI level seven weeks after the last injection of cortisone was only 1.7 gamma per cent, or 20 per cent of the control value of 5.9. A final sample was obtained on this patient 160 days after the end of therapy, at which time the PBI had returned to normal.

In 4 of the patients (Table 3) concomitant serum cholesterol and PBI

studies were made. The 2 patients (B.V. and M.T.) who demonstrated a significant fall in PBI levels had parallel rises in serum cholesterol levels to a significant degree. In the other 2 patients (A.S. and E.M.) the minimal change in PBI was likewise accompanied by little rise in total cholesterol concentration.

TABLE 3. CHANGES IN SERUM CHOLESTEROL AND PROTEIN-BOUND IODINE LEVELS DURING MASSIVE CORTISONE THERAPY

Patient	Protein-bound iodine (gamma per cent)			Total cholesterol* (mg. per cent)		
	Control	During treatment	Per cent change	Control	During treatment	Per cent change
M.T.	5.9	3.4	−42	170	268	+58
B.V.	5.3	4.1	−23	143	203	+42
A.S.	5.9	5.2	−12	178	182	+ 2
E.M.	3.6	3.3	− 8	182	208	+14

* Data of Dr. F. Stare.

Absence of thyroidal depression in patients with exophthalmic goiter

Because of the marked effect demonstrable on the euthyroid gland, 2 patients with classic exophthalmic goiter were selected for a trial of cortisone at the same dosage level.

N.F., a 16-year-old school girl had a nine-month history of tremor, prominence of the eyes, muscular weakness and swelling in the neck. Physical examination revealed a pulse of 130, B. P. 140/60, a flushed, warm, moist skin, exophthalmos with lid lag and decreased convergence, and a diffuse enlargement (3–4×) of the thyroid gland with palpable thrill and bruit. She had received some propylthiouracil prior to her admission, but without control of symptoms. Laboratory data: B.M.R. +40 per cent; I^{131} uptake over the thyroid in three hours, 96 per cent; serum PBI, 22.5 micrograms per cent; and serum cholesterol, 85 mg. per cent.

M.B., a 33-year-old housewife gave an 18-month history of tremor, heat intolerance, dyspnea, nervousness, protrusion of the eyes, and goiter. Physical examination revealed a pulse of 100, marked hyperactivity, emotional lability, exophthalmos with lid lag, tremor, and a diffuse enlargement (4×) of the thyroid gland with audible bruit. Laboratory data: B.M.R., +58 per cent; I^{131} uptake over the thyroid in twenty-four hours, 99 per cent; and serum cholesterol, 91 mg. per cent.

A dose of 500 mg. of cortisone daily was administered orally to each of these patients over treatment periods of seven days. At the end of this time the thyroidal I^{131} collection appeared unchanged in both patients, the curves of accumulation over the 24-hour period being practically superimposable over the pre-treatment curves (Fig. 6). There was no

effect on the basal metabolic rate or the clinical condition of either patient, although the PBI of one (N.F.) had shown a slight fall, from 22.5 to 17.7 gamma per cent.

DISCUSSION

These data appear to demonstrate a relatively constant effect of large doses of cortisone acetate upon the manner in which a tracer dose of

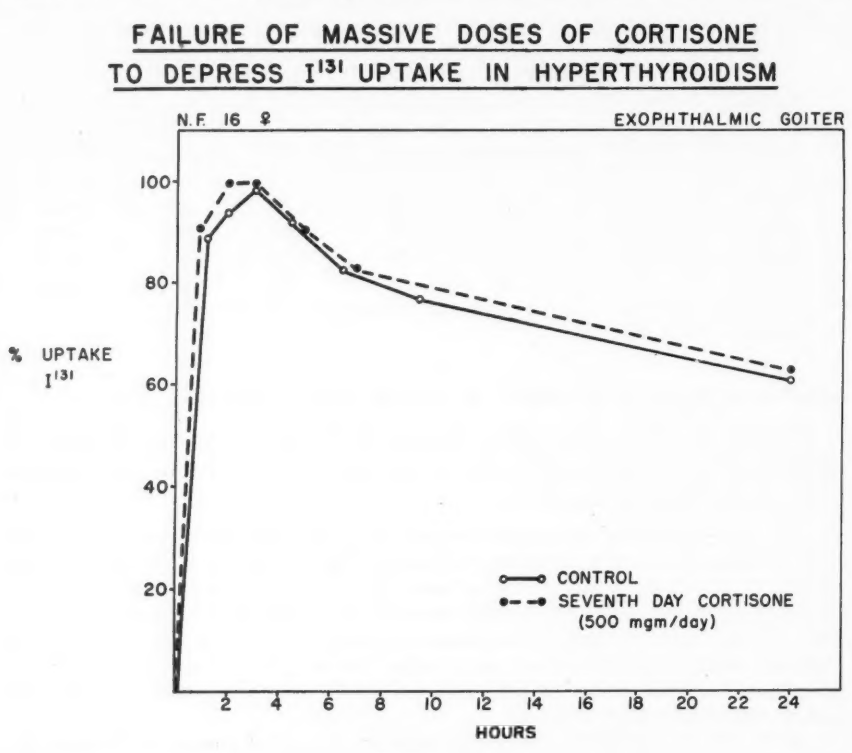

FIGURE 6

radioiodine is accumulated by the normal thyroid gland, as measured by an *in vivo* counting system. The depression in radioiodine accumulation may be striking in degree and is usually associated with a fall in the level of the serum PBI. It is apparent that this effect appears early after the onset of cortisone administration and that the time required for "recovery" of this function after cessation of therapy may be very short (1–2 days) if cortisone is given by the oral route, but prolonged (2–7 weeks) if the intramuscular route is employed.

Keating (12) and others have stressed the limitations inherent in the use of epithyroidal counts following a tracer dose of I^{131} as an index of the functional state of the thyroid tissue itself. An increase in the total body iodide pool could theoretically reduce by dilution the quantity of labeled iodide arriving in the thyroid, independent of any changes in thyroid function. However, direct measurement of changes in plasma volume during treatment with large doses of cortisone have not to date shown sufficient plasma dilution to account, on this basis alone, for the increase in iodide pool seemingly necessary to produce the observed changes in thyroidal uptake (13). Crude estimation of changes in body fluids were attempted in all patients by following total body weight, hematocrit, and total serum protein levels, although the tendency of anemia to improve and the concentration of serum globulins to fall during treatment in patients with rheumatoid arthritis reduced the significance of the determinations. The average gain in weight during therapy was 1.8 kilograms, the average fall in hematocrit was 4 per cent, and the average fall in the level of serum proteins, 12 per cent, all well below the magnitude of percentage change observed in radioiodine-accumulation (RAIU) or PBI determinations. The reduction in the quantity of iodide available to the thyroid gland through the production by cortisone of either an abnormal fixation of iodide to extrathyroidal tissues or an increased excretion of iodide through a primary renal effect of the steroid, remains to be excluded. Further work will be necessary to ascertain whether or not within the thyroid cell itself there is a selective effect on the trapping of inorganic iodide or its incorporation into the organic molecule. Likewise a change in the effect of the pituitary on the thyroid under cortisone must be considered. Woodbury, Ghosh, and Sayers have reported a decreased thyroidal accumulation of I^{131} in hypophysectomized rats given cortisone and TSH, as compared with similar rats given TSH alone (14). A final interpretation of these results rests upon clarification of the mechanism involved. Whatever the mechanism responsible for the changes in RAIU and PBI after cortisone, the net effect is compatible with a depression of thyroid function.

The failure of even these large doses of cortisone to alter the iodine accumulation of the hyperactive thyroid gland is in agreement with most of the previous unfavorable reports concerning treatment with moderate doses (100 mg. per day) (2, 15, 16).

Several investigators have reported a rise in the level of serum cholesterol after cortisone therapy (3, 17). The small series of observations made during this study have shown a close reciprocal relationship between the serum cholesterol and the PBI levels, and suggest that the increase in the former during cortisone therapy may depend primarily upon a decrease in the level of circulating thyroid hormone.

SUMMARY

In euthyroid patients, massive doses of cortisone acetate have been observed to decrease consistently the percentage of a tracer dose of I^{131} accumulated in the thyroid gland, accompanied in most cases by a significant fall in the level of serum protein-bound iodine.

The physiologic implications and the possible mechanism for this effect are discussed.

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ALTERATION OF THYROID FUNCTION BY ACTH AND CORTISONE*

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RECENT observations have indicated that both ACTH and cortisone produce an alteration in the function of the normal thyroid gland. Hill, Reis, Forsham and Thorn (1) have noted a decrease in the radioiodine accumulation gradient, a depression of the basal metabolic rate and a decrease in the serum protein-bound iodine concentration during ACTH therapy. Hardy, Riegel and Erisman (2) also noted a decrease in the level of the serum protein-bound iodine. Money *et al.* (3) observed a decrease in thyroid weight and thyroidal radioiodine accumulation in rats. Wolfson *et al.* (4) have confirmed the results reported by Hill, Reiss, Forsham and Thorn and used the term "corticogenic hypothyroidism" to designate these changes.

In an attempt to evaluate further the changes in thyroid function during ACTH or cortisone therapy, we have determined the 48-hour radioiodine excretion, the basal metabolic rate, and the levels of total and free serum cholesterol, before, during and after ACTH and cortisone administration.

METHODS AND PROCEDURES

Seven patients with rheumatoid arthritis, 1 with active rheumatic heart disease, 1 with gout and 1 normal subject were studied. Prior to the beginning of therapy the basal metabolic rate, serum cholesterol concentration and 48-hour radioiodine excretion were determined. Following the administration of ACTH (100 to 200 mg. daily) or cortisone (200 mg. daily) for ten to sixteen days, these determinations were repeated. Ten to fourteen days after the cessation of therapy the studies were again repeated.

The specimen for assay of 48-hour radioiodine excretion was obtained by the accurate collection of all urine voided for forty-eight hours after the oral administration of from 50 to 75 microcuries of I^{131} without added carrier. The amount of radioiodine present was determined by the use of a Geiger-Müller (thin mica window) counting tube and scaling circuit.

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The cortisone was purchased with funds granted by the United States Public Health Service. The ACTH was supplied by Armour and Company.

An aliquot of the administered solution of I^{131} was preserved and an appropriate dilution used as a standard. A 0.4-ml. portion of this standard and 0.4 ml. of the 48-hour urine collection were placed on separate filter papers in planchets, 0.4 ml. of concentrated NH_4OH containing a small amount of glue was added to each, and the solutions were evaporated to dryness by means of an infra-red lamp. The total counts present in each were then determined simultaneously in the counting apparatus, and corrected for "background," which was consistently about 0.5 count per second. No correction was made for the amount of dissolved solids present in the urine specimens. The results are expressed as the percentage of the administered amount of I^{131} which was present in the 48-hour urine collection.

Total and free cholesterol levels in serum were determined by a modification of the method of Schoenheimer and Sperry (5). All basal metabolic rates were determined by the same technician using a Benedict-Roth closed system apparatus.

RESULTS

Radioiodine excretion: In 7 of 10 patients studied there was a marked increase in the excretion of I^{131} during therapy with ACTH or cortisone, in 2 patients there was a slight increase, and in 1 there was a slight decrease (Fig. 1). The changes in mean values and standard errors are given in Table 1. During treatment with ACTH or cortisone, the differences from the pre-treatment values were statistically significant ($p = .01$). Following the discontinuance of therapy, there was a slight decrease from the pre-treatment value in 4 of the 10 patients, no change in 3, and an increase in 3. None of these latter changes is statistically significant, and the mean value corresponded closely with the mean value obtained before treatment.

Basal metabolic rate: No consistent change in the basal metabolic rate was noted. The rate decreased in 4 of the 6 patients studied and increased in 2. The mean value during treatment corresponded closely with the pre-treatment value. There was a slight decrease in the mean value of the BMR after discontinuance of treatment, although this was not significant.

Cholesterol: In the 8 patients in whom serial cholesterol determinations were carried out on serum, no consistent change was noted during either cortisone or ACTH therapy. In the 6 patients receiving ACTH, the serum cholesterol concentration increased in 2, remained unchanged in 2, and decreased in 2. In the 2 patients receiving cortisone, there was an increase in serum cholesterol concentration in one patient and a decrease in the other. Neither of these changes is statistically significant and they have not been included in the mean values shown, which apply only to ACTH-treated individuals.

DISCUSSION

The increase in radioiodine excretion occurring during the administration of ACTH and cortisone is consistent with the findings of other observers (1, 3, 4) of a decrease in thyroidal radioiodine accumulation in

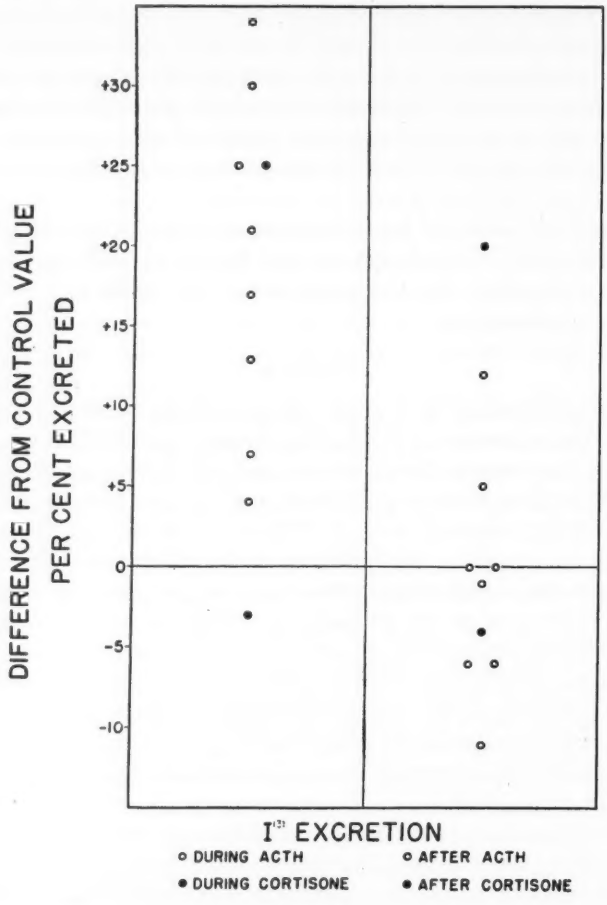

FIGURE 1

experimental animals and human subjects. Since the extra-thyroidal disposal rate of radioiodine probably influences the thyroidal total accumulation or accumulation gradient, the possibility cannot be eliminated that sufficient alteration of renal function by ACTH or cortisone may produce these changes. Significant increases in glomerular filtration rate

in patients receiving ACTH have been noted by Earle *et al.* (6) and by Ingbar *et al.* (7). The primary role of the thyroid, however, in determining the relative proportion of ingested radioiodine that is fixed in the thyroid gland is indicated by the observation of Thorn *et al.* (1) that the decrease in the thyroidal iodine accumulation gradient resulting from ACTH or cortisone administration is abolished by the administration of thyrotropic hormone. It would appear likely, therefore, that the increased radioiodine excretion noted by us is the result primarily of decreased accumulation in the thyroid. This probably results from a sup-

TABLE 1

Therapy	Patient	48-Hour I^{131} excretion (%)			B.M.R. (%)			Serum cholesterol (mg. %)					
								Before		During		After	
		Before	During	After	Before	During	After	Total	Esters	Total	Esters	Total	Esters
ACTH													
	U		95	61			−10					181	
	Go		82	57			+22					196	
	R	63	76	52	0	−1.5	− 4	198	155	258	201	220	158
	F	74	81	73	+12	+3	+ 2	178	139	179	131	220	166
	Ge	63	80	57	+12	+0.5	+ 9	258	202	232	170	254	190
	N	54	58	48	+17.5	+5	+17	220	160	218	156	286	204
	D	37	58	42	+ 1.5	+18	+12	244	186	208	157	246	178
	P	57	87	69	+ 1.5			166	124	177	134	214	164
Cortisone													
	V	47	72	67				212	152	316	215	282	196
	M	55	52	51	0	+12	0	231	174	174	126	211	153
Mean		56.8	74.1	57.7	+ 6.4	+ 6.7	+ 6.0	210.7*	161.0*	212.0*	158.2*	227.1*	176.7*
Standard Error		3.1	4.4	3.1	3.7	2.8	3.8	14.9*	11.8*	12.8*	10.5*	11.9*	7.2*

* ACTH-treated patients only.

pression of TSH production by the pituitary, as pointed out by Thorn and co-workers, or from a primary effect on thyroid activity, rather than from an alteration in renal function. The depression of serum protein-bound iodine (2) lends further support to this concept. Renal clearance studies of radioiodine excretion would, of course, be helpful in further elucidation of this problem.

The lack of change in the basal metabolic rate may be due to the "calorigenic" action of ACTH and cortisone. This has been noted by several observers (1, 4) and is further verified by the finding of Beierwaltes *et al.* (8) of an increase in basal metabolic rate in patients with myxedema during cortisone administration.

It is of interest that there was no consistent change in the serum cholesterol levels after ten to sixteen days of treatment in the 6 subjects who received ACTH. Conn (9) has noted a sharp decrease in serum cholesterol

levels after three to five days of ACTH administration, and Adlersberg (10) has observed a slight decrease after six to fifteen days followed by an increase upon further treatment. During cortisone administration an increase in serum cholesterol level has been noted (4, 10). It is possible that the lack of change in both esterified and free serum cholesterol concentrations observed by us during treatment with ACTH was due to the time interval chosen for measurement.

SUMMARY

Of 10 patients receiving ACTH or cortisone, a marked increase in 48-hour radioiodine excretion was observed in 7, a slight increase in 2, and a decrease in 1. The mean value returned to the pre-treatment level after the hormone was discontinued. No significant changes were noted in basal metabolic rate or serum cholesterol levels after ten to sixteen days of ACTH administration.

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PSEUDOHYPOPARATHYROIDISM: REPORT OF A CASE SHOWING BONY DEMINERALIZATION

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IN 1942, Albright and associates first described 3 patients with a condition which they called pseudohypoparathyroidism (1). Up to the present time, only 9 similar patients have been mentioned in the English medical literature (2–9). The findings in pseudohypoparathyroidism are very much like those in nonsurgical or "idiopathic" hypoparathyroidism. Characteristically they include tetany with low serum calcium and high serum phosphorus values, a tendency to early cataract formation, delayed dentition with faulty enamel, mental retardation, usually increased bone density, and frequently metastatic calcification in the cerebral basal ganglia. Most of the patients have had "spells," either epileptiform or tetanic in nature.

According to the present concept of the mode of action of parathyroid hormone, its administration to patients with a parathyroid deficiency should result in an increased phosphorus output in the urine. This phenomenon has been repeatedly demonstrated in idiopathic hypoparathyroidism (1, 2, 7 and 10–15). However, in pseudohypoparathyroidism the patients have failed to respond in any way to large intravenous doses of parathyroid hormone, either by an increased urinary phosphorus or by a change in the level of serum calcium or phosphorus. Certain other features common to most of this group of 12 patients described in the literature help to differentiate them clinically from idiopathic hypoparathyroidism. There is a tendency to shorter stature, shortening of the long bones and metacarpals, and rounding of the facial contour. Metastatic calcification in the basal ganglia and particularly in the skin is seen more frequently. There appears to be a greater incidence of convulsions and mental deficiency.

The limited postmortem and surgical material supports the thesis that pseudohypoparathyroidism represents not a deficiency of parathyroid hormone but rather a failure of end-organ response to the hormone. Thus, biopsy of the parathyroid glands in 2 patients with pseudohypoparathyroidism revealed the glandular tissue to be normal in one and hyperplastic

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in another (2). On the other hand, at necropsy in 3 patients with idiopathic hypoparathyroidism there was an apparent absence of parathyroid tissue in 2 instances (10, 16), and almost complete replacement by fat in another (17).

The following case history is presented as an example of pseudohypoparathyroidism with the unusual finding of bony demineralization.

CASE REPORT

K.P., a 29-year-old male Negro, was admitted to the Los Angeles County Hospital in April, 1948, because of drainage of two weeks' duration from an opening near his rectum. He was said to have been a normal full-term baby but mental retardation had been noted since kindergarten age. He did not progress beyond grade one, was unable to read or write, and had a limited vocabulary. He always had had an amiable disposition and was able to work as a shoe shine boy. His stature had always been less than that of other children of his age. First evidences of puberty were noted at about 21 years.

Since the age of 8 he had suffered from "spells" of variable frequency, described by the mother as a "stiffening of the muscles, jerking of the legs, and throwing back of the head." These lasted only a few moments and often caused him to fall, but he did not lose consciousness or control of his bladder or bowels. His muscles were always stiff, with frequent minor involuntary "jerkings" and he had poor control over them. Difficulty with vision had been noted since the age of 8, becoming much more severe in the two years prior to admission. Small hard lumps in the skin of the hands, neck, and back first appeared at the age of 10. These had slowly increased in number.

The family history was noncontributory. There was no external evidence of disease in the mother, father, or in the one male sibling, aged 24. The father's height was 66 inches (167.6 cm.), the mother's 61 inches (155 cm.), and that of the sibling, 70 inches (208.3 cm.).

On physical examination, the patient was short but fairly well proportioned. His measurements were as follows: height 60 inches (152.4 cm.), weight 116 lbs. (52.6 Kg.), span 57 inches (144.8 cm.), head circumference 22½ inches (57.1 cm.), chest circumference 30½ inches (77.5 cm.), abdominal circumference 29 inches (73.7 cm.), pubes to vertex 29½ inches (74.9 cm.), and pubes to floor 30½ inches (77.5 cm.). He obviously was retarded mentally and had a poor memory, but was cheerful and obedient. His hair distribution appeared normal and his secondary sex characteristics were well developed. The face was somewhat rounded. His gait was propulsive with a forward tilt and the legs and arms were held stiffly. The muscles were rather tense and there was some difficulty in extension of the fingers and hands and abduction of the thumbs. The tendon reflexes were hyperactive. The Chvostek and Trousseau signs were strongly positive.

The blood pressure was 118/75 mm. of mercury and the pulse 74. The eyes revealed bilateral lenticular opacities described as wedge shaped white-grey densities following the suture lines of the lens. The ears and nose were normal. Only 8 teeth were present—4 incisors, 3 bicuspids, and 1 molar—and neither the mother nor the patient could recall the loss of any teeth since early childhood. With the exception of the one molar, they were poorly developed and of small size. No abnormalities were noted in the heart, lungs, or abdomen. A draining rectal fistula was present. Over the dorsum of both hands, the back of the neck, and the sacral area, were clusters of small hard nodules which were in the skin and freely movable over the subcutaneous structures. Except for the features mentioned above, the findings on neurologic examination were negative.

The blood counts and the findings on urinalysis were repeatedly within normal limits. The blood Wassermann test gave negative results. The concentration of serum protein-bound iodine was 5.9 micrograms/100 cc. The 24-hour 17-ketosteroid excretion (method of Talbot) was 4.7 mg. (normal 9–22 mg.). The electrocardiogram revealed a markedly prolonged QT interval, together with a finding not usually associated with hypocalcemia—inversion of the T waves in the left ventricular precordial leads. The serum sodium concentration was 141 mEq./liter, the serum potassium 4.3 mEq./liter, the carbon dioxide combining power 30 mEq./liter, the serum chlorides 103 mEq./liter, the nonprotein nitrogen 30 mg./100 cc., the serum albumen 5.2 Gm./100 cc., and the serum globulin 2.5 Gm./100 cc. Serum calcium, phosphorus and phosphatase values were determined on numerous occasions. When the patient was untreated, the calcium levels were always low (5.8 to 7.0 mg./100 cc.) and the phosphorus levels high (5.4 to 9.2 mg./100 cc.). After treatment with dihydrotachysterol the serum calcium level ranged as high as 10.4 mg./100 cc. and the phosphorus level as low as 5.0 mg./100 cc. Alkaline phosphatase values were between 2.6 and 5.7 Bodansky units/100 cc. Blood sugar levels, measured at half-hour intervals after the ingestion of 100 Gm. of glucose, were 61 (fasting), 97, 87, 88, 65, 73, 72, 74, 67, 70, and 69 mg./100 cc. The blood eosinophil count was 97 per cu. mm., with a drop to 12 per cu. mm. four hours following a dose of 1.5 mg. of adrenalin given intravenously in 200 cc. of saline. Large volumes of urine of low specific gravity were excreted at all times but, by forcible restriction of water for an 18-hour period, urine of specific gravities of 1.024 and 1.022 was obtained. Phenolsulphonthalein excretion was 62 per cent in one hour, with an appearance time of three minutes. Intravenous pyelograms showed good dye concentration and the findings were interpreted as normal.

The reports of the roentgenograms were as follows: Skull: "The skull is normal in size and shape though there is considerable thickening of the bones of the calvarium which have a coarsely reticular trabecular pattern. The suture lines are open. There are bilateral symmetrically distributed flocculent calcifications throughout the region of the basal ganglia. In addition, numerous curvilinear calcifications are present bilaterally, their appearance suggesting that they are located in the walls of somewhat dilated lateral ventricles." Mandible: "Dental development is considerably retarded. The permanent bicuspids and molars lie in irregular fashion deep within the osseous structure of the mandible and only the incisors and cuspids have erupted." Chest: "The heart and great vessels are within normal limits. The lung fields are clear." Spine: "The entire spine is normal excepting for a mild degree of demineralization." Pelvis and long bones: "There is generalized demineralization, particularly of the lower ends of both femurs and the upper ends of the tibiae and fibulae, with considerable broadening of the medullary canals and marked thinning of the cortices. Multiple 'growth lines' are seen in the metaphyseal ends of these bones. There is moderate coxa vara deformity of both hips with shortening and thickening of the femoral necks and flattening of the femoral heads. Secondary osteoarthritic changes are seen about both acetabula. The pelvis is quite small and has a reticular appearance similar to that of the skull. All the epiphyses are closed." Hands: "The metacarpals and phalanges of both hands are short and thick and do not show the usual modeling. The most marked changes are seen in the 5th metacarpals." Feet: "The metatarsals and phalanges of both feet are fairly normal in contour and development but are markedly demineralized." Soft tissues: "Collections of granular cutaneous calcifications are present in the lower cervical regions bilaterally, over the dorsum of the lower lumbar and sacral areas, and scattered about the hands and feet."

Several tests for response to parathyroid hormone were made according to the tech-

nique of Ellsworth and Howard (13). On two occasions, following 200 units of parathormone given intravenously, and twice following 500 units, the patient failed to exhibit any phosphorus diuresis (Table 1). Initially no control was used. On the second test a control patient was selected who, unfortunately, was a diabetic; and though a moderate phosphorus diuresis followed the injection of the parathormone, it was felt that the results might be inaccurate because of the presence of diabetes. In the third test, thirteen months later, the control failed to exhibit a satisfactory diuresis, possibly because of the ingestion (against instructions) of a considerable amount of food at 11 p.m. on the night preceding the test. In the fourth test, using a new lot of hormone, a good phosphorus diuresis was obtained in the control with no demonstrable effects on the patient. No re-

TABLE 1. RESULTS OF THE ELLSWORTH-HOWARD TESTS IN PATIENT AND CONTROLS

Time	Urinary phosphorus, mg./hour						
	Nov. 4, 1949	Dec. 6, 1949		Jan. 29, 1951		Feb. 6, 1951	
	Patient	Patient	Control	Patient	Control	Patient	Control
7–8 a.m.	18.0	—	—	6.3	23.7	13.01	13.1
8–9 a.m.	4.8	6.0	10.0	18.8	16.2	13.1	14.9
9–10 a.m.	25.0	27.0	24.0	20.6	10.7	31.3	28.0
	Parathyroid extract, 200 units i.v.			Parathyroid extract, 500 units i.v.			
10–11 a.m.	15.0	5.0	56.0	6.3	27.8	19.8	109.2
11–12 noon	21.0	7.0	63.0	12.5	36.0	30.1	55.6
12–1 p.m.	16.0	10.0	16.0	12.5	15.7	31.8	44.9

actions were noted in either the patient or in the controls, and the results of skin tests on the patient, using a 1 to 10 dilution of the hormone intradermally, remained negative.

An electroencephalogram taken under partial barbiturate anesthesia at a time when the patient's serum calcium level approximated 10 mg./100 cc. was reported as showing no diagnostic abnormality.

On three different occasions therapy with dihydrotachysterol and calcium resulted in elevation of the serum calcium level, reduction in the muscle stiffness, and freedom from "spells." Though the patient felt better, he failed to keep clinic appointments.

The duration of the periods of therapy, the amount of the drugs used, and the response of the blood calcium level were as follows: From May 9 to May 17, 1948, he received 4 cc. of dihydrotachysterol and 12 grams of calcium lactate daily, with a rise in the serum calcium from 6.4 mg./100 cc. to 7.1 mg./100 cc. From November 13 to December 25, 1949, he received 4 cc. of dihydrotachysterol and 10 grams of calcium lactate daily with the serum calcium level changing from 6.2 mg./100 cc. to 8.5 mg./100 cc. On December 22, 1950, he was seen again with a markedly positive Chvostek sign (serum calcium level unknown). Between this date and January 23, 1951, he took 30 cc. of dihydrotachysterol, 300 tablets (5 gr.) of calcium lactate, and 100 perles (50,000 units) of vitamin D. These medications were taken in large amounts, without regard to the prescription, in an effort "to get well." On January 23, 1951, the serum calcium level was 10.6 mg./100 cc.

During the periods of therapy the patient's "spells" were relieved. On one occasion while in the hospital, prior to the institution of therapy (serum calcium level about 6.2 mg./100 cc.), he had a generalized tonic and clonic convulsion lasting about one minute, with loss of urinary sphincter control, apnea, and tongue biting. He remained stuporous for one hour following this episode, during which time he was given 10 cc. of calcium gluconate and 8 cc. of calcium chloride intravenously. Five minutes following the last injection he had another typically epileptiform convulsion lasting forty seconds and remained deeply lethargic for several hours. The mother stated that none of his other "spells" were of this nature.

A metabolic study was conducted from June 21 to July 15, 1951, at a time when the patient was presumably in a relatively stable state so far as calcium and phosphorus were concerned, having had no therapy for five months. A constant daily diet was consumed with calcium and phosphorus contents calculated from dietary tables. The calcium content was low (100 mg., calculated) and was supplemented by 5 grains of calcium lactate in powdered form three times daily. No food was rejected. Feces and urine were collected in two 3-day periods, before and after the administration of large doses of parathormone. Stool collections were timed by the use of carmine-red markers. Standard methods of chemical analyses were employed. There was no appreciable change in the calcium output in the urine or feces following parathormone. There was a moderate increase in urinary phosphorus (Table 2).

TABLE 2. RESULTS OF CALCIUM AND PHOSPHORUS BALANCE STUDY BEFORE AND AFTER PARATHORMONE

3-Day periods	Ca intake,* mg./24 hrs.	Fecal Ca, mg./24 hrs.	Urine Ca, mg./24 hrs.	Ca balance, mg.	Serum Ca, mg./100 cc.	P intake,‡ mg./24 hrs.	Fecal P, mg./24 hrs.	Urine P, mg./24 hrs.	P balance, mg.	Serum‡ P, mg./100 cc.	Parathormone, units/24 hrs.
1	230	—	—	—	5.8	700	—	—	—	6.0	—
2	230	—	—	—	7.0	700	—	—	—	5.7	—
3	230	206	4	†20**	6.8	700	237	277	†186**	—	—
4	230	—	—	—	7.4	700	—	—	—	—	600
5	230	—	—	—	6.4	700	—	—	—	—	600
6	230	198	†	†32**	5.8	700	232	338	†130**	6.2	900

* Calcium intake partially calculated from diet tables (100 mg.), plus 130 mg. from added calcium lactate powder
† Too low to measure accurately.
** Of questionable accuracy because of the calculated intake.
‡ Phosphorus intake calculated fram diet tables.

Operations for the rectal fistula (caudal anesthesia) and cataract extraction (sodium pentothal) were performed uneventfully.

DISCUSSION

The separation of nonsurgical hypoparathyroidism into 2 groups, "idiopathic" and "pseudo," appears to be justified on the basis of the different response to parathyroid hormone. Most of the cases reported as pseudohypoparathyroidism have repeatedly failed to respond to parathyroid extract, although at the same time the potency of the hormone has been proven by observations on control patients. The impor-

tance of the careful performance of the Ellsworth-Howard test is to be emphasized. The use of a control is essential since, as with any biologic preparation, the hormone may be of low potency. This is probably the explanation for our failure to obtain a satisfactory phosphorus diuresis in the control patients on two occasions (Table 1). In the application of the Ellsworth-Howard test to other hypoparathyroid patients and to normal subjects, we have not obtained as marked a phosphorus diuresis as did the authors of the test, though two different brands and several lots of parathormone have been employed.

Whether the clinical features mentioned are of any real value in differentiating the two groups of hypoparathyroidism may be open to question. Certainly there seems to be a much higher incidence of metastatic calcification in pseudohypoparathyroidism, particularly in the soft tissues. Skin calcification is mentioned rarely in idiopathic hypoparathyroidism. In 9 of the reported cases of pseudohypoparathyroidism, a description of the skin is included and of these, 6 had rather extensive calcification (2, 3, 4, 5, 7).

Similarly, intracerebral calcification appears to be more common in pseudohypoparathyroidism. It has been described in 4 of the 8 patients who had roentgenograms of the skull (2, 3, 4, 5), whereas the incidence is probably not this high in idiopathic hypoparathyroidism (18, 19, 20, 21, 26). Our patient, in addition to extensive basal ganglial deposits (Figs. 1a and b), showed linear calcifications, presumably in the walls of the lateral ventricles (Fig. 1b).

Shortness of stature has been noted in most of the patients with pseudohypoparathyroidism. Of the 7 patients whose height was mentioned, 6 were unusually short. However, some of the younger patients with idiopathic hypoparathyroidism are also described as being considerably shorter than the normal for their age.

Stockiness of build has also been considered to be characteristic of pseudohypoparathyroidism; our patient was repeatedly described as "stocky" until the nude photographs were examined (Fig. 2).

The moon face described in many cases of pseudohypoparathyroidism is a difficult quality to measure objectively, as is evidenced by the varying opinions expressed about our patient's facies.

Shortness of the metacarpal bones has not been described in the idiopathic form of the disease, possibly because it has not been looked for, whereas it has been noted frequently in pseudohypoparathyroidism. The suggestion of a hereditary component to the disease is provided by the finding of short metacarpals in the mother of the patient studied by Dr. J. S. L. Browne (7). Some shortening and thickening of the metacarpals is evident in our patient (Fig. 3), though it is not striking. The hands of

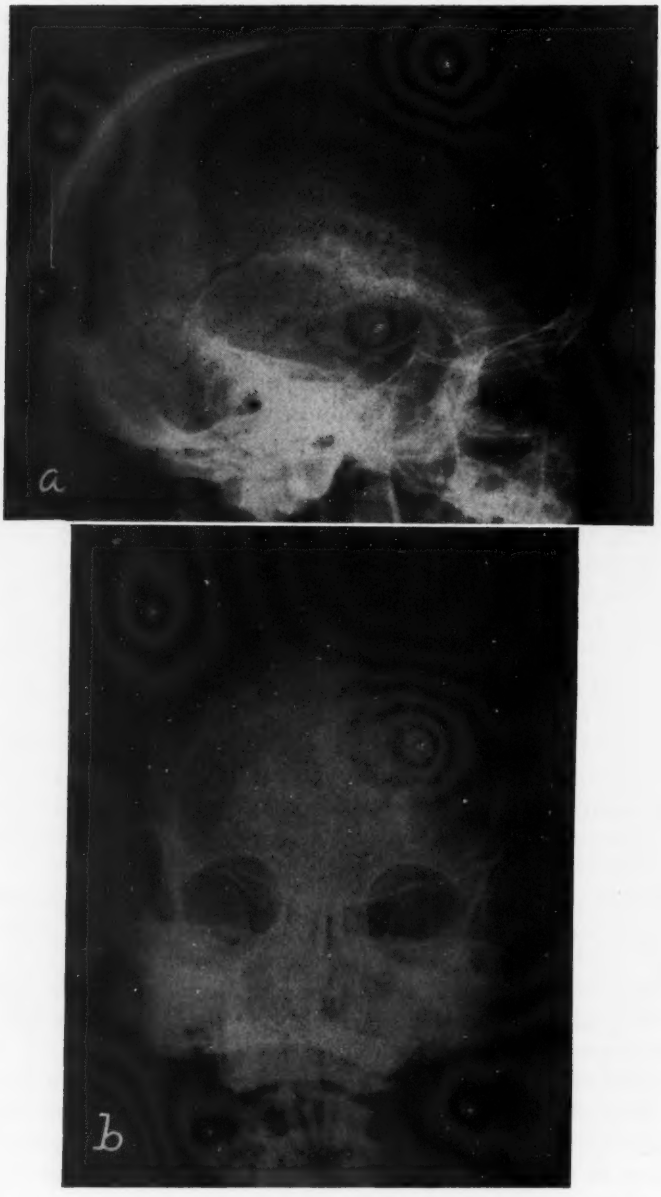

FIG. 1, *a* and *b*. Extensive symmetrical calcification in the basal ganglia. Unerupted teeth and curvilinear calcifications in the region of the lateral ventricles are evident in Fig. 1 *b*.

both parents and the sibling appeared normal; roentgenograms of the father's metacarpals showed these bones to be within normal limits.

The serum calcium levels of both parents were likewise within normal limits.

With the probable exception of the short metacarpal bones, most of the clinical and radiologic criteria just mentioned do not differentiate abso-

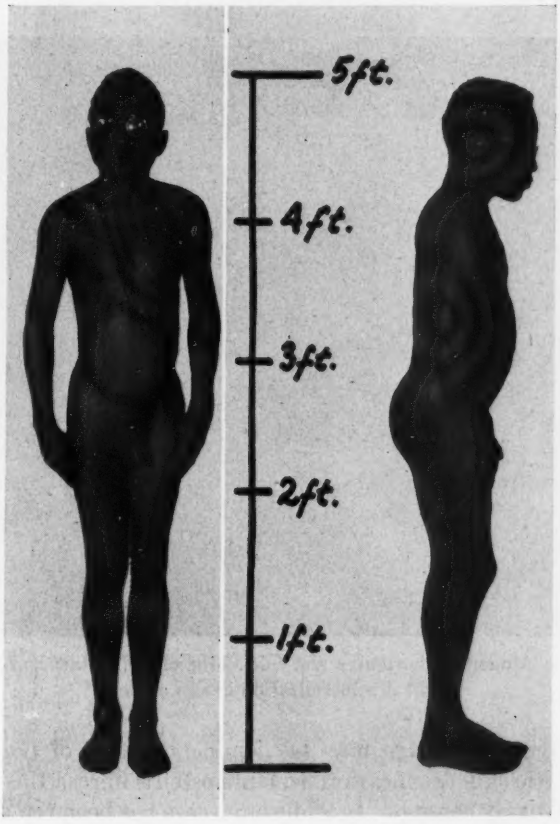

FIG. 2. Short stature but relatively normal proportions.

lutely between idiopathic and pseudo hypoparathyroidism. Possibly the increased incidence of some of these characteristics in the pseudo form may indicate that the latter is a more long-standing condition, present from birth or early childhood, rather than appearing late in life as a result of an infection or other disease process.

Considerable bony demineralization was evident in our patient, par-

ticularly in the long bones (Fig. 4), pelvis, spine and feet. Demineralization of bone was mentioned in one other patient with pseudohypoparathyroidism (4), and is said by Albright to occur occasionally in idiopathic hypoparathyroidism (2) (page 32). It is interesting to speculate on mechanisms that could provide for an increased tendency to calcification in some areas (brain and skin) while there is decreased calcification in others (bones and teeth). A similar paradox exists in other clinical entities, *e.g.*, in

FIG. 3. Moderate shortening and thickening of the metacarpal bones. Slight demineralization is also evident.

hyperparathyroidism there may be demineralization of bone coexistent with metastatic skin calcification. In renal osteitis fibrosa this same combination is relatively common. In addition, a case has been reported in which following removal of a hypertrophied parathyroid gland, metastatic skin calcification decreased markedly concomitantly with increased calcification in the skeleton (22).

Albright's explanation for the mechanism involved in hypoparathyroidism is contained in his textbook (2) (page 17). He believes that even though the calcium×phosphate ion product in the serum is constantly high, tending towards supersaturation, the local calcifying factors (mechanisms for phosphate ion increase) are less effective than in the normal

subject. Thus, deposition of lime salts in places like the teeth or even bone might be subnormal, while at the same time the tendency towards supersaturation of the serum would cause metastatic calcification in the brain and skin. The most obvious objection to this theory is that it fails to explain why the bones are usually more densely calcified than normal in hypopara-

FIG. 4. Marked demineralization evident in the lower ends of the femurs and upper ends of the tibiae and fibulae.

thyroidism and only rarely demineralized. Unless some other factor is involved, one would expect the bones to behave in a more uniform manner, as do the teeth.

The situation in this patient is highly analogous in many respects to that in renal osteitis fibrosa cystica. Hyperphosphatemia with presumably secondary metastatic skin calcification and bony demineralization are present. Parathyroid hyperplasia[1] may exist, as in one of the patients reported by Albright (2). Most authors appear to regard the bone lesions of renal osteitis fibrosa as a consequence of an increased output of parathyroid hormone, either by way of a direct action on bone or by some

[1] We were unable to obtain a parathyroid biopsy specimen.

unexplained secondary action. Albright, however, has expressed the opinion that the bone lesions are the result of the chronic acidosis found in this condition. Strong evidence for the former view is afforded by the findings in an undoubted case of renal osteitis fibrosa recently reported by Dreskin and Fox (22). In this patient, following removal of a large hypertrophied parathyroid gland, there occurred a marked drop in the level of serum calcium, together with healing of the bone lesion (as shown by biopsy) without any appreciable elevation of the serum phosphorus level and presumably with no change in the chronic acidosis. One could postulate then that in pseudohypoparathyroidism the inability of one end organ (kidney) to respond to parathyroid hormone could lead to parathyroid hyperplasia. Oversecretion of the hormone then might affect another more responsive end organ (bone) and cause demineralization.

In an effort to determine if oversecretion of parathyroid hormone might be responsible for the bony demineralization in our patient, calcium and phosphorus balances were measured during a control period and following administration of large amounts of parathormone. Though it had been previously determined that parathormone in moderate dosage had no effect on urinary and serum phosphorus and calcium concentrations, the possibility of an effect on fecal calcium excretion had not been eliminated. In the normal human, administration of parathyroid hormone does not appreciably increase fecal calcium (23). There was no significant change in serum, fecal or urinary calcium after a total of 6,300 units of parathyroid extract given in a nine-day period (Table 2). Serum and fecal phosphorus values were unchanged. There was a small increase in urinary phosphorus which, in view of the associated findings, was probably not enough to be of significance. The hormone used was of two lot numbers, samples of both of which had been shown to produce a considerable phosphorus diuresis in normal subjects. It is probably justifiable to conclude that parathormone had no effect on calcium metabolism in this patient and, therefore, that parathyroid hyperplasia was not the cause of the bony demineralization present. There are other possibilities that are not entirely excluded: 1) The parathormone in the individual vials used in the test may have been inactive, even though the samples tested (from the same lot numbers) were active. 2) It is conceivable, though unlikely, that in this patient resistance may have developed to injected hormone because of its previous use in the Ellsworth-Howard test (a total of 14 cc. over a period of eighteen months). The available evidence in the literature and our own experience do not support this possibility. 3) Perhaps endogenous parathyroid hormone is maximally active, maintaining the blood calcium at a higher level than it would otherwise assume and perhaps contributing to higher than normal fecal calcium excretion

and to deposition of calcium in the skin and brain. If this were so, it might be reasoned that the addition of even a large amount of exogenous parathyroid hormone would not give any additional detectable response. However, the amount of parathormone administered to this patient was several times that usually required to maintain normal calcium levels in a completely parathyroidectomized human.

Another possible cause for the bony demineralization is a long standing mildly negative calcium balance brought about by a combination of a poor calcium intake and a high fecal excretion. High fecal excretion of calcium apparently is present in renal insufficiency (24, 25), probably because of the high level of blood phosphate and consequent precipitation of calcium phosphate complexes in the intestine. Several patients with renal disease described by Lin and Chu (24) were in negative calcium balance on intakes of from 4.7 to 20.7 mEq. of calcium daily. On higher intakes of calcium (about 60 mEq. daily) the balances became positive. Because of the high blood phosphorus level, a similar mechanism for intestinal precipitation of calcium phosphate probably exists in hypoparathyroidism. With this mechanism, plus a diet poor in calcium, chronic negative calcium balance might result. Though accurate information was not available, it seemed that our patient's calcium intake was most likely relatively low. Milk was rarely purchased at his home and he had difficulty in masticating leafy vegetables. However, an appreciably negative calcium balance was not evident, even during the relatively low calcium intake of the balance period. Unfortunately, too much reliance cannot be placed on this phase of the balance study, since a portion of the calcium intake (food) was calculated rather than analyzed. A more prolonged balance study with varying intakes of calcium and phosphorus would have been of interest but was not obtainable.

Because of the persistent polyuria, it was at first considered that renal or posterior pituitary disease was present in this patient. The polyuria, however, appeared to be due to a firmly entrenched habit of drinking large volumes of water, since, when fluids were forcibly restricted for eighteen hours, satisfactory urine concentration was attained. All other tests of renal function gave normal results.

The oral glucose tolerance curve was quite flat rather than diabetic in type, as in the patient reported by Moehlig and Gerisch (8). An intravenous test was not obtained.

Except for moderate depression of 17-ketosteroid excretion on one occasion, there was no evidence of dysfunction of the endocrine glands.

In patients described in the literature, with either form of hypoparathyroidism there is often confusion as to whether the "attacks" they complain of represent epilepsy or tetany. Definite epileptiform convulsions

have been described, though it is likely that brief generalized tetanic muscular contractions are more commonly the basis for the complaint. Our patient suffered from numerous attacks of tetany, coming on with blood calcium levels around 6.0 mg./100 cc., and completely relieved by administration of dihydrotachysterol. In addition, he had at least one typical epileptiform convulsion which was not controlled by intravenous calcium.

SUMMARY

A 29-year-old male Negro with pseudohypoparathyroidism is described. This is the thirteenth such patient to be reported. The findings included typical blood chemical changes, tetany, mental retardation, cataracts, basal ganglial and skin calcification, delayed eruption of the permanent teeth, mildly shortened metacarpals, and at least one attack of epilepsy. An unusual finding was considerable generalized demineralization of bone. Parathormone was shown to be without measurable effect on calcium or phosphorus metabolism. Possible mechanisms for the bony demineralization are discussed but no definite conclusions reached.

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Endocrine Review

PRACTICAL ASPECTS OF INSUFFICIENCY OF THE ANTERIOR PITUITARY GLAND IN THE ADULT

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A VOLUMINOUS literature bears witness to the intense interest and attention centered on the pituitary gland, and to the position of prime importance that it occupies among the endocrine glands. Such notoriety, though perhaps justified, has introduced many divergent opinions with sufficient confusion to distract the interest of the average reader from certain practical data relating to pituitary disorders.

It is not difficult to understand how this situation has evolved: 1. Conclusions based on animal work have been too freely transposed and applied to human beings. 2. The tools used in studying pituitary function, especially in man, have been notably crude. 3. Many pituitary hormones employed clinically have been impure, of varying potency and quite unpredictable in the response which they elicit. 4. Inaccuracies have been perpetuated by adherence to historical precedent. Cachexia often is still regarded as a *sine qua non* of destruction of the anterior pituitary, probably because it was mentioned in the early descriptions of Simmonds' disease. 5. The diagnosis of endocrine disease has often been based on flimsy evidence. Thus, to some, every fat child with girdle obesity and small external genitalia becomes an instance of Fröhlich's syndrome, and every woman with menstrual irregularity has a primary pituitary endocrinopathy.

The basic physiology and biochemistry of the anterior pituitary have been excellently summarized in books, such as those of Van Dyke (1) and Cameron (2), and in the publication of lectures and discussions presented at conferences (3). *Recent Progress in Hormone Research* (4) and the annual volumes *Vitamins and Hormones* (5) are especially valuable. An excellent brief summary which relates research in the fundamental sciences to the care of the patient was written by Young (6). Farquharson's monograph (7) and McCullagh's review (8) are outstanding. The most recent reports are to be found in current issues of *Endocrinology*, the *Journal of Clinical Endocrinology* and other journals.

We shall concern ourselves with matters of purely clinical interest and with observations rather exclusively confined to man and shall limit

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ourselves to those patients who have, or are supposed to have, an insufficiency of the anterior pituitary. We shall not discuss in this article those diseases associated with pituitary hyperfunction, such as acromegaly.

First a word regarding terminology. We cannot see much to choose between the terms "pituitary insufficiency" and "panhypopituitarism"; we prefer the former term, since "panhypopituitarism" implies a total lack of all pituitary hormones, a condition but rarely found. We do, however, object to the term "Simmonds' cachexia" and "pituitary cachexia," since cachexia is so rarely a part of pituitary insufficiency (9). Common usage forces us to perpetuate such a term as "Simmonds' disease." If any man's name should be perpetuated by such association, then "Sheehan's syndrome" should be used to describe postpartum necrosis of the pituitary. The difficulty, and the common denominator, is that these conditions were all described long before the publication of the papers which link personal names to these medical conditions. Falta (10) early described pituitary insufficiency under the term "multiple ductless glandular sclerosis"; included are patients suffering from "Simmonds' disease," "Sheehan's syndrome," and so forth.

We shall not separate "pituitary insufficiency" into various subgroups such as "pituitary myxedema," "pituitary adrenal failure" and "pituitary hypogonadism." We shall discuss pituitary insufficiency only as it is seen in adults. Dwarfism is a separate subject. Finally, we shall not discuss Fröhlich's syndrome, because we do not know what the condition is, but we do not believe that it represents primary pituitary insufficiency.

Undoubtedly we shall be criticized for the omission of certain references. We have tried to include those articles which in themselves give an adequate bibliography. We cannot see any reason to relist the 462 references in Escamilla and Lisser's review (11), the 307 references in Sheehan and Summers' review (12), nor the 921 references in Friedgood's review (13). Nor do we wish the readers to assume that we have critically analyzed all the data summarized or that we agree with all the authors' diagnoses or advice. Rather we have tried to present a review with as little personal bias as possible.

The review by Escamilla and Lisser (11) is quite complete. They accepted only 101 of the published cases as true Simmonds' disease. Our work as reviewers has been made much simpler by the publication in 1949 of a complete summary by Sheehan and Summers (12). We shall quote extensively from this important contribution. They have re-evaluated the 101 cases referred to at the beginning of this paragraph and have accepted only 37 as involving severe pituitary lesions of long duration and uncomplicated by hypothalamic pressure. They questioned Escamilla and Lisser's four cardinal characteristics: 1) loss of weight, 2) loss of

sexual function, 3) asthenia and 4) a very low basal metabolic rate. (Not all patients with these symptoms have pituitary insufficiency, and certainly not all patients with pituitary insufficiency have all these symptoms.) Sheehan and Summers have accepted only cases with "concrete evidence of the destruction of the anterior pituitary." They have excluded all cases in which destruction of the pituitary has occurred terminally, as from metastasis or miliary tuberculosis, or from surface involvement in purulent or tuberculous meningitis.

CLASSIFICATION OF LESIONS

Sheehan and Summers found a great variation in the severity of the lesion "from negligible lesions up to almost complete destruction of the gland." They grouped their cases under five main sections which we shall present in abbreviated form.

1. *Acute lesions.*—An acute lesion is nearly always a necrosis of the anterior pituitary. The extent of the necrosis varies, and the duration of the acute stage is a matter of days or, at the most, a few weeks. At this stage there are no obvious symptoms of hypopituitarism. In the later healed stage there will be pituitary deficiency related to the amount of parenchymal loss. By far the commonest cause (79 of the 132 recorded necroses), and the condition which leads to the largest necroses, is severe circulatory collapse in an obstetric patient. The usual cause of this circulatory collapse is shock and hemorrhage at about the time of delivery. These necroses are not fatal in themselves and heal subsequently, leaving a small scarred anterior lobe. Hemorrhage into the anterior pituitary has only rarely been reported. When it was in any way gross, the accounts are always strongly suggestive of bleeding into a degenerating adenoma.

2. *Subacute lesions.*—The cases in this section are those in which it seems probable that the patient died within a few months after the onset of the pathologic process. In a few of the cases the hypothalamus was involved as well as the pituitary; such involvement is probably always fatal in the early stages and thus does not give rise to chronic lesions. In most of the cases, however, the lesion was confined to the pituitary and might well have healed to a scar if the patient had survived longer. There are also giant-cell granulomas involving the pituitary with or without involvement of the hypothalamus. Finally, there are scattered reports of miscellaneous lesions such as actinomycosis, chronic abscesses and so forth.

3. *Chronic fibroid lesions.*—This is a very large series of cases, showing various degrees of long-standing scarring, fibrosis and atrophy of the pituitary. The lesion is sometimes confined to the anterior lobe, and in other cases involves the entire gland. It is very difficult to arrive at a satisfactory classification for many of these cases. Some appear to be slowly

progressive fibroses of uncertain origin. Others appear to be the late healed stage of acute or subacute conditions which occurred many years previously; in these cases practically all evidence of the nature of the original lesion has disappeared, and only a small scarred gland is left. However, there is one large group (the postpartum necroses) in which the original lesion can be identified from the history. The remaining cases have been subdivided chiefly according to their histologic characteristics. Some of these groups are probably homogeneous in origin; in others there may have been a variety of etiologic factors leading to the same pathologic appearance. A number of dwarfs have been reported in whom the anterior pituitary is very small and fibrous with numerous cysts of the middle lobe. These cases, which will not be discussed further, are probably due to developmental abnormalities of the gland. In all, thirteen separate groups of chronic fibroid lesions are considered.

4. *Surgical hypophysectomy.*—There have been only 2 reported instances in which a normal pituitary was removed; once for the treatmentof diabetes and once for epilepsy. Hyperglycemia was controlled in the diabetic patient, but tuberculosis unfortunately developed and the patient died six months later. The second patient lived for six years, and pituitary insufficiency developed. Necropsy showed that the hypophysectomy had not been complete (14).

5. *Cysts and tumors.*—This section includes a large group of intrasellar lesions, such as simple cysts, cholesterol cysts, and tumors, usually adenomas or angiomas. Then there are the extrasellar lesions, including cysts (cysts of Rathke's pouch and craniopharyngiomas) and tumors.

RELATIONSHIP OF PITUITARY LESIONS TO OTHER ORGANS

Sheehan and Summers (12) then discussed the relationship of the degree of pituitary destruction to the structure and function of the other endocrine glands. Briefly, they reported that in 76 of 82 cases of severe pituitary insufficiency in which complete necropsy findings were available there was marked atrophy of all the other endocrine glands.

1. *Adrenals.*—The adrenals are small and thin. The remaining cortical parenchyma is very atrophic, but is not fibrotic. It contains a normal amount of lipoid, and probably represents mainly zona fasciculata. The zona glomerulosa is obscured by a great fibrous thickening of the capsule. The zona reticularis has usually disappeared completely; it is replaced by a broad layer of fibrous tissue which forms a sharp boundary between the cortex and the medulla. The medulla remains almost unaffected, and thus appears very prominent in sections. The combined weight of the two glands in 26 cases averaged 4.7 Gm. with a range of 2.2 to 7.8 Gm.; the range for the normal adult is 10 to 15 Gm.

2. *Thyroid.*—The thyroid is small; in 28 cases it had a mean weight of 7 Gm. with a range of 2 to 11 Gm., as compared with the normal weight of 20 to 30 Gm. Histologically it shows a variety of appearances. (*a*) Most commonly (26 cases) there is a well marked atrophy of the acini. (*b*) Frequently (19 cases) a more severe lesion is present; there is extensive fibrosis. (*c*) At other times (15 cases) the fibrosis becomes so severe that the thyroid is represented only by a small mass of dense scar tissue.

3. *Gonads and genital tract.* Female.— The essential lesion is an atrophy of the ovaries, and a superinvolution of the uterus, vagina and vulva. The changes are similar in type to those occurring after the menopause (though greater in severity). In women less than 45 years of age there are only 12 cases in which detailed descriptions of the ovaries are available. The combined weight of the ovaries was 3.8 to 5.4 Gm., as compared with the normal 10 to 15 Gm. A few primordial follicles were sometimes found, but there were never any developing graafian follicles or corpora lutea. The uterus was small. The cervix formed only a small dimple in the vaginal vault; the vagina and external genitalia showed advanced atrophy of the senile type.

Male.—The testes are usually described as very atrophic. The seminiferous tubules have enormous hyaline thickening of the basement membrane, which may almost obliterate the lumen. Sometimes they have no epithelial lining; sometimes they have a single layer of epithelium with no trace of spermatogenesis. The interstitial tissue is increased and consists of edematous hyaline connective tissue, and the interstitial secretory cells have disappeared completely. The epididymis, seminal vesicles and prostate are usually noted to be atrophic, but no details of the histologic changes have been recorded.

4. *Other organs.*—There is a striking atrophy of most of the viscera. This appears to be a true trophic atrophy, since nearly all the patients are of normal weight.

CLINICAL FINDINGS

As has been mentioned previously, the clinical findings of pituitary insufficiency vary with several factors, the most important being the degree of pituitary destruction. In some instances the amount of the pituitary destruction may be slight and the symptoms vague; in others, the diagnosis is obvious to the physician as the patient passes on the street. The pattern of the physical findings is related to the lack of hormones from the "peripheral" endocrine glands. One patient may suggest Addison's disease, another may strongly suggest myxedema, and a third may have few physical signs. The incidence of diagnosis is largely determined by the physician's "suspicion index." If suspected, pituitary insuffi-

ciency may be diagnosed; and it should always be thought of in a woman, previously well, who at the time of delivery has a circulatory collapse (often following a hemorrhage) and who, from that moment on, is a "different" individual from what she had been previously. The first symptom noted is usually an absence of lactation. The breasts become soft and involuted, and there is no secretion of colostrum or milk; later the breasts become small with inconspicuous areolae. The only other early symptom

FIG. 1. Two patients with pituitary insufficiency. Patient between them, with erroneous diagnosis of "Simmonds' cachexia," has anorexia nervosa.

is that, the patient's pubic hair having been shaved for the delivery, the growth of hair is slow and slight.

In most of the postpartum patients, there then follows the gradual development of chronic ill health. The "classic" patient will have amenorrhea, atrophy of the gonads and genital tract, loss of libido, almost complete loss of pubic and axillary hair, absence of normal cutaneous pigment, particularly obvious around the nipples, and lack of response to test sun irradiation (Sheehan and Summers, 1949) (12). The patient has a characteristic flabby facies, with thinning of the eyebrows and pallor of the cheeks. There is absence of sweating of the skin, as well as loss of the normal greasiness of the axillae. The patient has an increased sensitivity to cold, is physically weak, lacks ambition or interest, is often mentally slow, and usually talks with a slow, soft speech. Two such patients are seen in Figure 1.

Let us repeat that this description cannot apply to every patient, and let us re-emphasize the great differences in the histories and findings. The histories vary with the etiology; certainly the history given by a patient whose insufficiency is secondary to an adenoma of chromophobe cells is in contrast to that of the patient with postpartum necrosis. The clinical findings may be identical if the degree of insufficiency is the same. A roentgenographic examination of the sella turcica and a check of the visual fields may be necessary to enable one to establish the correct diagnosis.

Having described a "typical" patient, we should like to list the important findings as found in the patients on whom Sheehan and Summers reported (12):

1. *Sex function.*—Complete and permanent loss of sex function is an invariable consequence, whatever the origin of the pituitary lesion; there is no need to tabulate the figures. In women, menstruation always ceases. When the pituitary lesion develops slowly, there may be a period of infrequent and scanty menstruation before complete amenorrhea develops. In cases in which the matter was investigated, there was a striking absence of the general symptoms which accompany the natural menopause. Information about the sex function in men is available for only 7 patients, all of whom were impotent and had no libido. The libido was noted in only 14 women; it was lost in 13.

2. *Hair.* Body Hair.—In both sexes loss of the pubic and axillary hair together appears to be of considerable diagnostic importance. Loss of axillary hair alone is not significant. The usual course is that the axillary hair is lost in a few months, but the pubic hair may persist for a year or two, and scanty short hairs may remain on the labia majora almost indefinitely. However, in patients who had had the pubes shaved at the time when the pituitary was destroyed, there is commonly a clear history that the hair did not grow again. The retention of pubic hair may be taken as an indication either that the pituitary lesion is not severe, or that it is not of more than about two years' duration.

Eyebrows.—All the patients examined by Sheehan and Summers (12) had considerable thinning of the eyebrows. The loss was particularly marked over the outer part, as in myxedema, and any hair remaining in this position tended to be depigmented.

Head Hair—About half the patients are recorded as having had some thinning of head hair, and the other half as having had normal or thick head hair.

3. *Skin.*—The skin is nearly always recorded as dry, and is usually described as smooth and soft on the face and body, but sometimes very slightly scaly on the limbs. The dryness of the skin is due to an absence of sweating; in 31 of 32 cases with details on this point there was no recog-

nizable sweating. There has been a lack of the normal greasiness of the axillae, as well as an absence of emotionally conditioned axillary sweating.

Striking pallor of the face is often noted; the color is often described as waxy white or alabaster-like. In 16 patients it is recorded as sallow or as having a lemon-yellow tinge; these were usually patients whose clinical appearance suggested myxedema. None of the patients was described as pigmented. There are two separate considerations regarding the pallor of the skin; namely, loss of cutaneous pigment and loss of the capillary flush.

a. The loss of cutaneous pigment appears to be due to a true interference with melanin production. The mammary areolae were always depigmented. Exposure of these patients to real or artificial sunlight produced no pigmentation in the irradiated areas. (This can be used as a diagnostic test, but it is necessary to take great care in attempting to pigment these patients because of the severe sunburn which may occur.) In the erythema ab igne which is almost always present on the legs of the female patients, brown pigment does not develop.

b. The other feature of the pallor is the lack of the normal capillary flush. This flush is completely absent in most cases, although the mucosae are normally colored.

4. *Facies.*—Most of the patients, particularly the women, have a characteristic and almost pathognomonic facial appearance. The facial contours tend to appear weak, suggesting what might be described as a partial regression of those features which are normal in adults and become coarsened in acromegaly. There is a lack of animation in the face, giving an expression suggestive of myopathy or of a person asleep. Slight puffiness of the face, particularly below the eyes, is commonly present. The picture is completed by the thinning of the eyebrows and the striking pallor of the skin. These appearances are in some ways suggestive of myxedema. In the literature, 33 patients are described as of hypothyroid appearance and only 3 as being not of hypothyroid appearance. Some of the patients had quite a definite appearance of myxedema; there are 12 such cases recorded.

Since Simmonds' original description, considerable attention has been directed to progeria as a typical feature of hypopituitarism. Nevertheless, an appearance of premature senility has been noted in only 10 per cent of the cases.

5. *Cardiovascular system.*—Roentgenologically the heart is usually small, in contrast to the enlargement which is sometimes seen in myxedema. Electrocardiographic findings were noted in 20 cases. In 13 cases low voltage electrocardiograms were recorded; in 4 there were negative T waves in Leads II and III; irregularity of rhythm was recorded in 3 cases. The changes are very similar to those of myxedema, and treatment with thyroid may produce a return to normal. The patients tend to have a

labile blood pressure. The mean systolic pressure was 108 mm. of mercury; 34 patients had a systolic blood pressure between 70 and 95, 45 between 100 and 135, and 11 between 140 and 240. The majority of the low blood pressures recorded were from patients in the last two or three months of life, and most of the higher blood pressures were in patients several months or years before death.

6. *General symptoms.*—Many of the symptoms are suggestive of myxedema. Patients complain of being sensitive to cold, and, as a result, sit by the fire as much as possible, so that erythema ab igne is commonly observed in women. The absence of sweating has been discussed. Rheumatism, particularly of the knees, was noted in 13 patients. The speech was recorded in 44 cases as slow, quiet, monotonous and rather hesitating. In 10 of these, chiefly in the terminal phases, it was noted as thick or slurred. The voice is usually soft and gentle. Mental torpor is very common; the patient, having no initiative, loses interest in her home and friends, and settles into a state of placid inertia. Patients are content with life wherever they are kept, being equally happy in their homes or in a hospital. Definite mental changes, usually depression or melancholia, were noted in 22 patients.

DIFFERENTIAL DIAGNOSIS

From Figure 1 and from the foregoing description, it is obvious that pituitary insufficiency may be confused with many other conditions. This will be referred to again under laboratory studies but here, from the standpoint of clinical differentiation, we should list the following diseases:

Diseases to be distinguished from pituitary insufficiency

1. *Nephrosis.*—Some patients with pituitary insufficiency may have the edema and coloring of a patient with nephrosis. The patient in Figure 2 had nephrosis with no evidence of pituitary insufficiency.

2. *Severe anemia.*—Occasionally there is marked anemia, and most of the patients referred to us (and some of our own) have been treated for months or years for "pernicious anemia" or for "stubborn anemia." The only way to eliminate this diagnosis of the cause of the trouble is to suspect pituitary insufficiency.

3. *Myxedema.*—This mistake in diagnosis is easily understood, for many of these patients do have myxedema. They may have every clinical aspect of primary myxedema (Fig. 3), including the facies, the voice, the delayed reflexes, and so forth. Even the results of laboratory studies, including an elevated serum cholesterol level, may be compatible with the diagnosis of primary myxedema; and we are sure that in the experience of others, as in ours, there are patients being treated for myxedema alone

who have "pituitary myxedema" as described in detail by Means and his associates (15), by Thompson and his associates (16) and by others (17, 18, 19). The only way in which to make the correct distinction is to think of both conditions, to have an "increased suspicion index." We have made an erroneous diagnosis often enough that we now make it a rule that in every patient with myxedema we always inquire into the possibility of

FIG. 2. Patient with nephrotic syndrome, before (left) and after (right) treatment.

postpartum necrosis and always obtain a roentgenogram of the sella turcica to exclude a tumor.

4. *Addison's disease.*—This is less frequently confused with pituitary insufficiency than are the foregoing conditions, but we have had several patients in our own experience whom we have treated for years as having Addison's disease, who were later found to have pituitary insufficiency. Again this mistake is not surprising, since in some patients there are clinical and laboratory changes characteristic of Addison's disease. Some authors rather glibly assume that the differential diagnosis is easy because patients with pituitary insufficiency are pale (and in general this is true) and patients with Addison's disease are pigmented (and in general this is true), but there are enough exceptions to warrant the statement that in making one diagnosis the physician should always exclude the other.

We have had 2 patients with pituitary insufficiency who had pigmentation suggestive of Addison's disease.

5. *Miscellaneous.*—Some patients with pituitary insufficiency have such marked hypoglycemia, particularly in the coma of a severely ill patient, that they are suspected of having hyperinsulinism. Study reveals that the hypoglycemia is but a part of the syndrome. Because of their mental changes, patients are sometimes reported as having a primary psychosis

FIG. 3. Primary myxedema, before (*a*) and after (*b*) treatment.

or as having had encephalitis; or the amenorrhea may receive full attention; and, unless suspected, the pituitary insufficiency may remain undiagnosed. Hemochromatosis may involve so many of the endocrine glands as to reproduce the syndrome of pituitary insufficiency.

6. *Anorexia nervosa.*—Finally there is anorexia nervosa, first described by Gull (20). We have saved it for the last because it has no connection with primary pituitary lesions. It is an anorexia usually occurring in young women, caused by a deep-seated psychic disturbance. As the result of insufficient food the patients lose weight, sometimes to a remarkable degree, and secondarily develop amenorrhea, low basal metabolic rate and other signs, symptoms, and results of laboratory studies, which lead to the

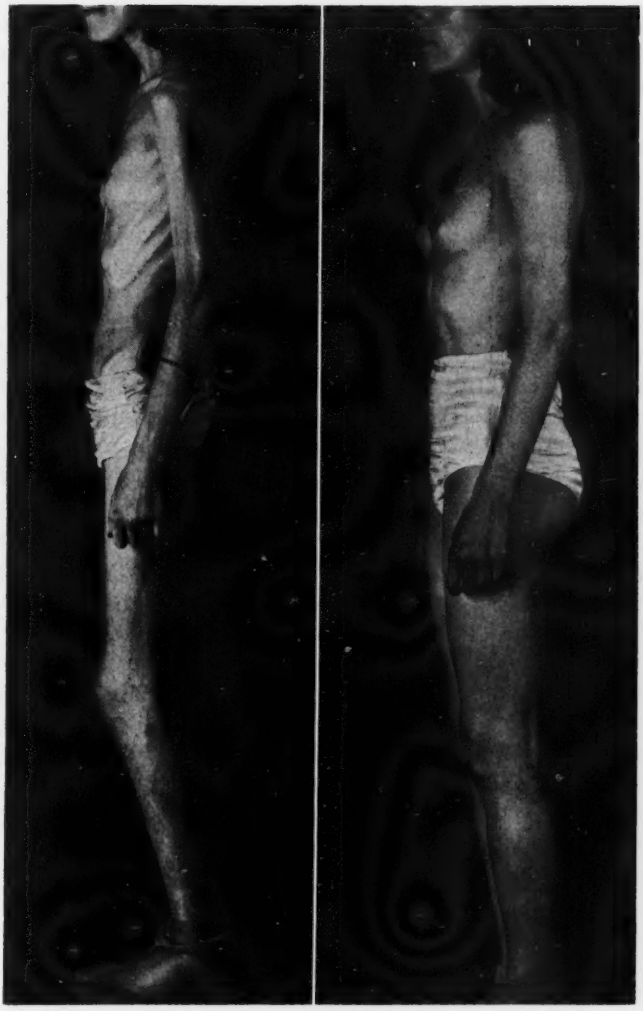

FIG. 4. Anorexia nervosa, before (left) and after (right) treatment with diet alone.

erroneous diagnosis of pituitary insufficiency. For the sake of argument, let us state that most of the patients reported as having "Simmonds' cachexia" really have anorexia nervosa; a critical review of the literature will support this statement. As a matter of fact, Sheehan and Summers (12) stated: "The nutritional state in this control sample of patients dying without pituitary disease was thus worse than in the patients who had

died from severe pituitary lesions." Any time a physician reports a cure of "Simmonds' cachexia" from the injection or oral (!) administration of pituitary hormones, one can be sure that the patient had anorexia nervosa and was helped by psychotherapy. Figure 4 shows the results before and after treatment of anorexia nervosa by Berkman (21), using only psychotherapy and a diet with increasing numbers of calories. If these patients will co-operate and if they want to get well, they can do so and all their glands will function normally without receiving a single "shot." Ryle (22) and Stephens (23) have published helpful articles on this subject.

Laboratory procedures useful in differential diagnosis

There are several laboratory procedures which have been used as aids in the diagnosis of pituitary insufficiency. Paschkis and Cantarow (24) recently reviewed their laboratory studies in patients suffering from hypopituitarism. The best known and most widely used are the basal metabolic rate and blood cholesterol values as an index of thyroid function; studies of the utilization of water and chloride as an index of adrenocortical function; the assay of urine for 17-ketosteroids, corticoids, estrogens and pituitary gonadotropic hormones (25); and the study of the patient's response to insulin and glucose. We shall also refer to observations following the injection of thyrotropic hormone and studies with tracer doses of radioiodine. These tests alone or in combination have been used in the study of pituitary insufficiency and of those conditions which resemble it, such as anorexia nervosa, Addison's disease, myxedema, primary hypogonadism, and marked debilitating disease. It should be obvious that no one can plan a chart of laboratory studies which can be "filled in" and the diagnosis determined. Yet an analysis of multiple studies may lead to the proper diagnosis.

Several workers have studied the effect of various metabolic disturbances on the circulating blood and, since the use of ACTH, this subject is receiving more attention (26–30). De la Balze and his associates (31) have listed detailed blood counts in patients with pituitary insufficiency. They studied total leukocyte, the neutrophil and the lymphocyte levels in the blood of patients with Cushing's syndrome, patients with Addison's disease and patients with pituitary insufficiency. The changes in the last two groups were quite similar and were the antithesis of the findings in Cushing's syndrome.

Carbohydrate metabolism is affected by many hormones and by the metabolic and nutritional status of the body. The overproduction and underproduction of the hormones from the pituitary, thyroid, pancreas, adrenal cortex and adrenal medulla, and the metabolic state of tissues such as the liver and muscles will affect the response of the body to

glucose and to insulin. This whole subject of carbohydrate metabolism has been recently reviewed by Soskin and Levine (32). Fraser, Albright and Smith (33) reported their excellent studies on pituitary insufficiency and various conditions which can be confused with it.

1. *The fasting blood sugar.*—It is important to remember that some patients suffering from pituitary insufficiency or from Addison's disease may have severe hypoglycemia as an outstanding finding. One of our patients with severe pituitary insufficiency was found to have a blood sugar concentration of 30 mg. per 100 cc., having been unconscious for more than eighteen hours before being brought to the hospital. Balfour and Sprague (34) have discussed the association of diabetes mellitus with disorders of other endocrine glands and Feldman and his associates (35) have reported the coincidence of diabetes mellitus and hypopituitarism.

2. *The sugar tolerance curve.*—This is not the place for a detailed discussion of the various methods used and the various interpretations given. Again we must refer to the literature (33, 36). There is a decreased sugar tolerance curve (hyperglycemic unresponsiveness) in diabetes mellitus and in some patients with a hyperfunctioning lesion of the adrenal cortex or anterior pituitary. An increased sugar tolerance curve (hypoglycemic unresponsiveness) has been frequently described in patients suffering from pituitary insufficiency and it is reported less frequently in anorexia nervosa, myxedema and Addison's disease. In the opinion of Sheehan and Summers (12) the sugar tolerance curve is not of value in the diagnosis of pituitary insufficiency.

3. *Insulin tolerance tests.*—These involve the study of the patient's response to injected insulin—both the rapidity and degree of drop in the amount of blood sugar (insulin sensitivity) and the rate and degree of rise (responsiveness to hypoglycemia). Fraser, Albright and Smith (33) have published charts of the type of curves obtained in myxedema, as compared with normal individuals and patients suffering from pituitary insufficiency. They reported that the curves for patients with Addison's disease did not drop so precipitously but the hypoglycemic unresponsiveness lasted longer. *Warning: Many patients suffering from pituitary insufficiency or Addison's disease can have extremely severe reactions to insulin. Sheehan and Summers (12) have records of 5 patients who died following the injection of insulin. Patients should be given one third the usual dosage of insulin (0.03 unit per kilogram body weight instead of the usual 0.1 unit per kilogram).*

There are other tests utilizing combinations such as glucose and insulin, insulin and epinephrine, and glucose and epinephrine. No test should be prescribed unless certain conditions are fulfilled, particular attention being paid to the importance of a high-carbohydrate diet for several days before the test (36).

Fraser and Smith (37) expressed the belief that the insulin tolerance test is the only one which will separate the myxedema of pituitary insufficiency from the myxedema of simple thyroid deficiency. In their opinion this distinction cannot be made by assaying the urine and its hormone content, since there are too many exceptions. They stated:

> Thus myxoedema, while giving in some cases indistinguishable results by the 17-ketosteroid assay, gives a characteristically different insulin tolerance curve. Therefore, by the use of this test, no case of primary myxoedema should be mistaken for panhypopituitarism. A raised serum-cholesterol level was found in many of the panhypopituitary cases, and so this feature of myxoedema cannot be used to differentiate primary myxoedema from panhypopituitarism . . . .
>
> With eight cases of anorexia nervosa it was found that four gave a normal result with the insulin tolerance test, but that in three of the remainder it was similar to that found in panhypopituitarism. With three cases of primary myxoedema this test showed a slow initial fall or insulin resistance, which differentiated them from all the cases of panhypopituitarism . . . .
>
> It is concluded that by the combined use of the tests, panhypopituitarism (anterior), producing even fairly mild symptoms, can be demonstrated and distinguished from allied syndromes. The insulin tolerance test distinguishes primary myxoedema, and anorexia nervosa is distinguished sometimes by both, and, in all cases tested, by the 17-ketosteroid assay. It is pointed out that clinical examination is also needed to exclude certain disorders, notably Addison's disease and severe liver disease.

Greenblatt and Kupperman (38) studied the response of many patients to the administration of glucose and insulin and found considerable variation in the curves. It should be emphasized that there are a great many factors which will influence a patient's response both to the glucose tolerance and insulin tolerance tests. For example, when Slessor (39) gave repeated insulin tolerance tests to the same patient he found a marked variation.

4. *Other laboratory procedures.*—Stephens (40) has described two other laboratory observations in patients having pituitary insufficiency. The first is the remarkably low basal metabolic rates, which are often at the "myxedematous level"; the second relates to studies of adrenal function in pituitary insufficiency as compared to myxedema, normal controls and patients with Addison's disease. Such studies of adrenal function are important in two ways: first, as an aid in the differential diagnosis and second, because of the fact that the physician should appraise the patient's adrenal function before beginning treatment. *Warning: Patients with adrenal insufficiency, whether due to Addison's disease or to pituitary insufficiency, are likely to be very sensitive to thyroid and may die following its use.* This will be discussed further later.

If facilities are not available for the study of chloride utilization, then adrenal function can be studied by the more easily performed water test (41, 42), or by studying the eosinophil response to an injection of ACTH

(43). It should be emphasized that such tests should not be considered specific. We are referring particularly to the fact that the water test is rather nonspecific and is affected by the status of renal function. Studies of adrenal function have been urged as a means of separating the adrenal insufficiency secondary to pituitary insufficiency from that which is secondary to myxedema or anorexia nervosa. Stephens (44) felt that this study of adrenocortical function would be specific in aiding in the differential diagnosis. Certainly Despopoulos and Perloff (45) would not agree with

TABLE 1. URINARY 17-KETOSTEROIDS. HYPOTHETICAL 24-HOUR VALUES*

	mg.
a) Normal male	14.0
b) Normal female	9.0
c) Female with Addison's disease	0.0
d) Male with Addison's disease	5.0
e) Panhypopituitarism (either sex)	0.0
f) Gonadectomized male	9.0
g) Gonadectomized female	9.0

* From Fraser, R. W.; Forbes, A. P.; Albright, F.; Sulkowitch, H., and Reifenstein, E. C., Jr.: *J. Clin. Endocrinol.* **1**:234, 1941 (50). Courtesy of Charles C Thomas, Publisher.

this, for they obtained the same results in patients with myxedema as in those with pituitary insufficiency.

Sayers and Sayers (46) reviewed the pituitary-adrenal system, and more recently Hill and others (47) evaluated the effect of ACTH and cortisone on thyroid function and followed thyroid and adrenal activity in various states. In evaluating these conditions and relationships they utilized various tests including radioactive iodine uptake, to be discussed later. Statland and Lerman (48) have studied the function of the adrenal cortex in myxedema, together with some observations on pituitary function. They divided myxedema into five groups, from primary myxedema to pituitary myxedema, in which Lerman has been interested for so many years. Knowlton and associates (49) have also recently reported on the effects of ACTH in pituitary insufficiency and myxedema. They did not find marked metabolic changes in a patient with pituitary insufficiency following injections of ACTH, nor were the metabolic changes in a patient with primary myxedema affected materially by treating the myxedema with thyroid.

Fraser and his associates (50) reported 1,500 urinary assays in which they studied the excretion of 17-ketosteroids and in some instances the quantity of the follicle-stimulating hormone. In general they found agree-

ment with the hypothetical values listed in Table 1. They found the excretion lowered by many nonendocrine conditions, notably malnutrition, various gastro-intestinal diseases, anemia, infections and particularly hepatic disease. They felt that this assay might help to separate patients with panhypopituitarism from those with anorexia nervosa "in which the values may be normal or lower but as yet not zero in any case." They found the 17-ketosteroid excretion very low in myxedema, "in rare instances less than 0.5 mg. per 24 hours." They felt that the assay of the urine for the follicle-stimulating hormone was of value in separating pri-

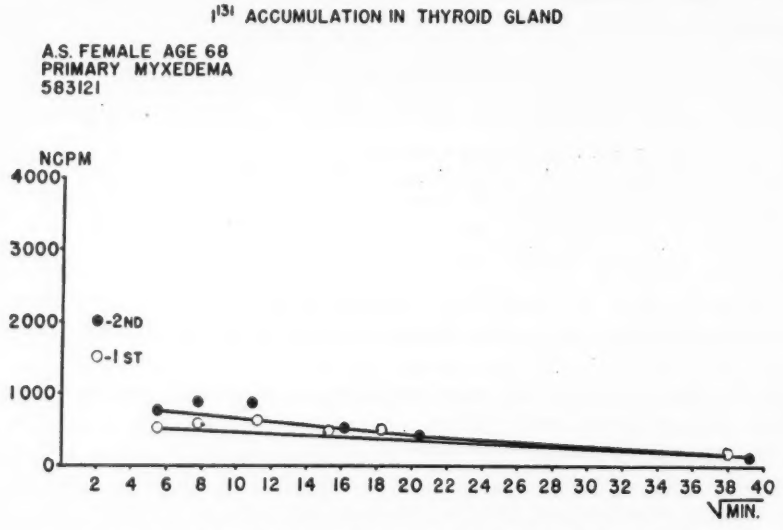

FIG. 5. Querido and Stanbury's curves of radioactive iodine uptake. Circles—before TSH; black spots—after TSH.

mary hypogonadism from hypogonadism which is secondary to pituitary insufficiency. The difficulty with this test, as with the others, is that it has not been found specific for any condition, and other observers have described the exceptions which are always found to any rule.

Engstrom and Mason (51) have also studied 17-ketosteroid excretion in patients with myxedema, and Klinefelter and his associates (52) have reported their observations on the excretion of the follicle-stimulating hormone.

In 1936 Bulger and Barr (53) reported data on 2 patients suffering from pituitary insufficiency whom they studied with the injection of thyrotropic hormone (TSH). In 1 patient the basal metabolic rate rose from −28 to −3 per cent, with other signs of metabolic improvement. In the second case no great effect was observed. In 1937 Scowen (54) reported that the

thyroid-stimulating hormone could be used to differentiate pituitary insufficiency from myxedema. Spence and Witts (55) in 1939, treated 2 patients who had pituitary insufficiency with this hormone. In the first patient they were able to raise the basal metabolic rate from −21 to +57 per cent, although they questioned the accuracy of the latter reading. In the second patient they raised the basal metabolic rate from −27 to +8 per cent but without the clinical improvement noted in the first patient.

Recently Querido and Stanbury (56) found that, in general, patients suffering from primary myxedema showed no response to the injection of TSH (Fig. 5), whereas 5 of 6 patients with pituitary insufficiency showed a positive response, as shown in Figure 6. This is an important observation

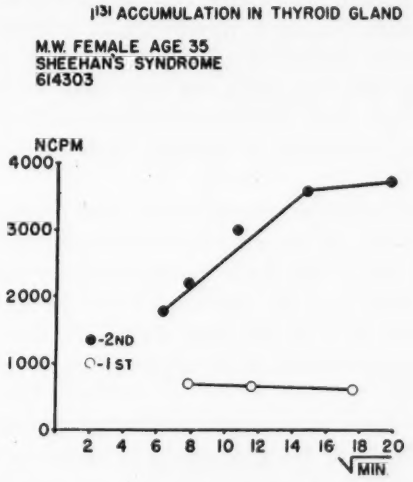

FIG. 6. Radioactive iodine uptake in pituitary insufficiency. Circles—before TSH; black spots—after TSH. (Figs. 5 and 6 from Querido, A., and Stanbury, J. B.: *J. Clin. Endocrinol.* **10**: 1192, 1950 (56). Courtesy of Charles C Thomas, Publisher.)

but it should be emphasized that 1 patient in this series of pituitary insufficiency had a response characteristic of primary myxedema.

The use of radioiodine in tracer doses may prove to be a valuable aid in making a differential diagnosis. Keating and his associates (57) reported that, in 46 cases of myxedema, there was a marked decrease in the uptake of radioiodine. Three patients with anorexia nervosa reported from the Mayo Clinic and 6 reported by Werner and his associates (58) in New York all had a normal uptake of radioiodine.

Comment on differential diagnosis

The question of greatest practical importance with respect to pituitary insufficiency is that of its clinical differentiation from anorexia nervosa.

From what we have written, one might conclude that these two conditions can readily be separated from one another. However, several of the differences enumerated are more apparent than real. A review of reported cases indicates that the symptomatology, physical signs and laboratory findings in pituitary insufficiency are often exactly duplicated by those of anorexia nervosa (22, 59, 60, 61). Berkman (21) has expressed the opinion that many of the symptoms of patients with anorexia nervosa are caused by a functional depression of the pituitary and that the clinical features may therefore be quite similar to those of pituitary insufficiency. Stephens (23) said: "The changes which occur in undernutrition may be regarded as expressions of a protective mechanism to compensate for the reduced caloric intake, mediated principally through a functional limitation of pituitary activity, with secondary hypofunction of those glands depending on the hypophysis for maintenance of normal structure and function." Sheldon and Young (62) expressed the view that "whatever may be the explanation of the flat curve in Simmonds' disease, it is at any rate clear that there is no need to invoke an endocrine explanation in its presence in anorexia nervosa."

Atrophic and degenerative changes have been produced in the anterior pituitary of lower animals by undernutrition (23, 63). This response to inanition is considered to be of the same nature as that occurring in the alarm reaction in human beings described by Selye (64), and is reversible in that it can be corrected by adequate diet. Keys and his associates (65) have completed a monumental study of the effect of starvation on human beings.

We have described the clinical signs and symptoms usually encountered in pituitary insufficiency. By contrast, the classic example of anorexia nervosa is represented by a nulliparous woman, usually younger than 30 years of age, in whom a history may be elicited of lifelong maladjustment or else of psychic trauma immediately preceding the onset of her illness. On examination, emaciation is often extreme; an increased growth of hair may be noted on the face, trunk and extremities; and, considering the degree of emaciation present, the degree of mental and physical activity displayed is surprising. If the patient will co-operate, dietary measures, often with supplementary psychiatric therapy, will usually rescue her from the disastrous effects of continuing starvation and restore her to normal health. If the patient does not respond to such treatment she may live for many years as "a living skeleton" or she may actually starve herself to death.

Between these two opposing pictures, there is a broad intermediate zone in which many of the reported cases lie. They incorporate several of the supposedly characteristic features of each condition, occasionally rendering clinical differentiation most difficult. There are those differences in response

to laboratory tests which are referred to, but there are too many exceptions to make even a "battery" of tests foolproof. Nothing can supplant the importance of a detailed history and a careful physical examination which will enable the physician to make the correct diagnosis 9 out of 10 times. The laboratory studies may not help with the tenth patient.

Other conditions which must be differentiated from pituitary insufficiency include myxedema and Addison's disease, as mentioned in the discussion of laboratory procedures. Common to pituitary insufficiency and myxedema are the low basal metabolic rate and changes in the skin and hair. In pituitary insufficiency the loss of hair occurs largely in the pubic and axillary regions. Both patients who have pituitary insufficiency and those who have Addison's disease will exhibit asthenia, hypotension, hypoglycemia and evidence of adrenocortical insufficiency. In pituitary insufficiency, however, cutaneous pigmentation is rare.

The state of nutrition in pituitary insufficiency is worthy of further mention. Escamilla and Lisser's (11) article listed cachexia as one of the four cardinal features of this disease. Everywhere in the literature the terms "Simmonds' disease" and "pituitary cachexia" are used interchangeably. Sheehan and his associates (66) however, in reviewing the nutritional state in 103 published cases with postmortem evidence of Simmonds' disease and in 55 living patients with this disease, found undernutrition or emaciation in only 13 to 27 per cent of instances. The disparity in figures between those recorded on the living patient and those found in the necropsy room strongly suggests that, when emaciation does occur in cases of pituitary insufficiency, it is frequently a terminal event. It is, of course, the usual finding in anorexia nervosa.

TREATMENT

The best way to treat a condition caused by hormonal insufficiency is to replace the missing hormone. This not-too-profound statement emphasizes our greatest deficiency in the treatment of pituitary insufficiency. Most of us will agree that the use of anterior pituitary extracts in the long-term treatment of human beings with pituitary insufficiency has been unsatisfactory (67). We have reason to believe that some of the time the preparations were not potent; when potent, they usually lost their potency in human beings, perhaps because of the development of antihormones (68–71).

Let us hasten to qualify this statement by saying that we are well aware of the important observations being made with thyrotropic, lactogenic, adrenocorticotropic and other hormones. We have mentioned the use of thyrotropic hormone in differential diagnosis and the studies in progress to determine its effectiveness in making thyroid tissue and metastases from carcinoma of the thyroid more receptive to the uptake of radioactive iodine

(72). Everyone is aware of the experimental and therapeutic uses of ACTH (73). The results and implications are most exciting. But we are not concerned with such studies and observations here, since the title of this paper is "*Practical* Aspects of Insufficiency of the Anterior Pituitary Gland in the Adult." And there are two discouraging features: the first is that all hormones so far isolated from the pituitary have proved to be protein in nature, and no one thus far has synthesized a protein hormone. We have available only the amount of hormone obtained from the pituitaries of animals, a fact which has limited the supply and caused the price to be high. The second discouraging factor is the one already mentioned; namely, the development of "antihormones" by the patient. We join with all other physicians in our confidence that somehow these problems will be solved, but not in time to be included in this review!

Since we have no hormones from the pituitary for routine treatment we have had to rely on the use of pituitary-like hormones and on the hormones of the other endocrine glands. Since pituitary insufficiency causes the loss of normal function of so many of these glands it is sometimes hard to know where to begin. This decision varies with each patient, but it is safest to consider adrenal insufficiency first. This has been mentioned before, but it deserves emphasis. Most of these patients have actual adrenal insufficiency, and it should be suspected in all others. They are particularly sensitive to thyroid and insulin. Insulin is rarely necessary for treatment, since so few patients have both pituitary insufficiency and diabetes (33). Such patients resemble, in some respects, the "Houssay dog." Insulin is, however, used in diagnostic tests. As mentioned earlier, it should be administered most cautiously and in small amounts. When hypoglycemia is a troublesome symptom the patient should follow a high-protein diet with perhaps six feedings a day. This type of program has been well described by Conn (36).

From a theoretical standpoint the best hormone for the treatment of adrenal insufficiency as part of pituitary insufficiency should be ACTH or cortisone. ACTH has been used to study a few patients; but, so far as we know, it has not been used for long-term treatment. Before discussing cortisone we should remark on the great improvement which many of these patients have had following the use of the types of replacement therapy found of value in the treatment of Addison's disease. To date the best extract has been the lipo-adrenal extract because it contains the highest percentage of the hormone which sustains the blood sugar level. This extract has the obvious disadvantage of being very expensive. In some cases the condition is controlled with desoxycorticosterone acetate. Although this is the less expensive form of treatment, it is also the most incomplete, since it effects only the retention of salt and water. If too much is administered it causes edema varying from slight pitting of the ankles to

generalized anasarca and heart failure. Thorn's (74) monograph thoroughly describes the treatment of Addison's disease and can be applied to many of these patients.

Cortisone has been a valuable addition for the treatment of these patients. They are much improved both subjectively and objectively; and, even at present prices, the cost in the average case is less than that of lipoadrenal extract. As the cost of cortisone decreases its availability will, of course, increase. At the present time physicians are having the opportunity to study combinations of cortisone with desoxycorticosterone acetate and with other hormones.

We cannot emphasize too frequently the inadvisability of administering thyroid to any of these patients until the physician is certain that any adrenal failure has been controlled. Many patients with pituitary insufficiency and pituitary myxedema are remarkably improved by taking thyroid. It is probably best to begin with a small amount such as ½ grain (about 0.032 Gm.) of uncoated desiccated thyroid daily. If there is evidence of adrenal insufficiency, cortisone should be administered before starting the use of thyroid. In most of these cases the condition is eventually controlled with the administration of 2 grains (about 0.13 Gm.) of thyroid or less per day.

Further "end-organ replacement therapy" involves the use of male or female sex hormone. It is possible with estrogens, with or without progesterone, to reproduce the bleeding cycle in the female. This treatment is well described in standard textbooks (75–80). Virtually all men and many women are markedly improved by the use of androgens, and our impression is that methyltestosterone is more effective than testosterone propionate. This has been the experience of Lisser (81) and others (66, 82, 83). A woman should receive the usual discussion before androgens are administered, being warned that, if androgens are used in large enough doses over a long enough period, she may observe hirsutism, which disappears with the discontinuance of the hormone, and lowering of the voice, which is permanent. The theoretic value of the androgenic hormones is not so much from their function as sex hormones as from their metabolic or anabolic properties. Hurxthal and Younghusband (84) have suggested that in some instances sex hormone therapy may cause a reactivation of chromophobe adenomas. We have had a similar impression in a few unpublished cases at the Mayo Clinic.

We should like to illustrate replacement therapy by recounting the treatment of 2 patients. The first is the treatment outlined by Dr. F. R. Keating, Jr., for a man seen at the Mayo Clinic.

This man registered first in December, 1948, at which time he was 54 years of age. He had always felt well until one year previously, when severe headaches, generalized ach-

ing, stiffness, weakness and exhaustion had developed. The stiffness and weakness were so pronounced that he could hardly get out of bed in the morning. Our first diagnosis was myxedema, but further study showed the sella to be enlarged by an intrasellar tumor, with extension toward the right. At no time were there any changes in the ocular fundi or visual fields. Roentgen therapy was administered, and replacement therapy was begun. Treatment was started on January 6, 1949, with 10 mg. of methyltestosterone by mouth three times daily, 2 cc. of lipo-adrenal extract by hypodermic injection daily and, later, 2 grains (about 0.13 Gm.) of desiccated thyroid (Parke, Davis & Company). His improvement was quite prompt and by January 17 he was referred to his home physician on the foregoing program with the addition of 2 Gm. of sodium chloride daily.

He returned on April 25 feeling "generally improved." "The stiffness was 75 per cent gone," and his "strength had improved 100 per cent." His beard was getting heavier and growing faster. His blood pressure, which had been 104 mm. of mercury systolic and 72 diastolic, was now 142 systolic and 88 diastolic. The prostate, which had been virtually absent, was now "about two thirds of normal size." He was told to decrease the amount of thyroid to 1½ grains (about 0.1 Gm.) and the lipo-adrenal extract to 1 cc. daily. In place of methyltestosterone we prescribed crystalline testosterone by buccal absorption, 10 mg. three times daily.

He returned in August to report further improvement. The 1 cc. of lipo-adrenal extract had not proved sufficient, and the dose had been increased to 1.5 cc. The improvement mentioned in the preceding paragraph was even more marked. His blood pressure was 136 mm. of mercury systolic and 76 diastolic. His skin seemed normal and was well tanned There was now normal axillary, pubic and facial hair. He was sent home without any change in his treatment. In December there was some difficulty with the customs authorities in sending crystalline testosterone into Canada, and he returned to the use of methyltestosterone by mouth.

He returned to the clinic on January 23, 1950, to report that he had adjusted his program until he was then taking 1½ grains (about 0.1 Gm.) of desiccated thyroid, 30 mg. of methyltestosterone, 3 Gm. of sodium chloride, 1 cc. of lipo-adrenal extract and 30 grains (about 2 Gm.) of aspirin daily. He had felt so well that he had married on January 13! There were no complaints except for some fibrositis which he still had in the cervical region. His blood pressure was 120 mm. of mercury systolic and 80 diastolic at this time. Dr. Keating discontinued administration of aspirin.

We did not see the patient from then until December, 1950. During these eleven months he had not changed his program in any respect. He was naturally quite elated because his wife was pregnant. He was anxious to know whether we could change the medicine, since it was rather an expensive program. His blood pressure was 132 mm. of mercury systolic and 74 diastolic, and the results of his general examination were essentially negative. Dr. Keating decided to discontinue administration of the lipo-adrenal extract and see whether 25 mg. of cortisone three times a week would constitute adequate replacement therapy. If so, this would save, at present, about $1,400 a year, since the cost of the lipo-adrenal extract was $130 a month. We have not seen the patient since that time but learned by mail that he was continuing to feel well.

There have been no great changes from the standpoint of laboratory studies. He never did have anemia, and his differential blood count was always essentially normal. The results of urinalysis were always negative. There had been no significant changes in the concentration of blood urea, uric acid, sugar, bilirubin, calcium, phosphate or phosphatase. The level of blood cholesterol, which was 234 mg. per 100 cc. in December, 1948, was 160 mg. per 100 cc. in January, 1950. There was no significant change in any of his

roentgenograms. There is record of only one determination of 17-ketosteroids, in December, 1950, at which time the reading was 1.7 mg. per twenty-four hours. His basal metabolic rates were −25, −26 and −27 per cent in December, 1948, but have been normal at −6 to −10 per cent in 1950.

Sheehan and Murdoch (85) have presented data on a most interesting series of 7 patients who fulfilled all criteria for postpartum pituitary insufficiency having developed, and who then became pregnant. One cannot imagine better indirect evidence of the healing of an infarct. A second point of great significance is the marked improvement which these patients had when they again became pregnant.

One of these patients is most interesting. When she was 30 years of age she was delivered of her fourth baby, and at that time suffered from necrosis of the pituitary with the characteristic findings of pituitary insufficiency. When she was 32 years of age she became pregnant for the fifth time, and during her pregnancy she improved dramatically. She changed from her vegetative existence to one of normal activity. This change was sustained, and the following year, when 33 years of age, she had her sixth baby with no complications. When, however, at 35 years of age she had her seventh baby she suffered from a postpartum hemorrhage and again characteristic pituitary insufficiency developed; but a year later she again became pregnant and had another normal delivery. It is of interest that with this pregnancy she had for the first time no improvement in her symptoms. One year later, when she was 37 years of age, she became pregnant for the ninth time, had a severe hemorrhage at the time of delivery and died. Interestingly enough and not unexpectedly, the postmortem examination of the anterior pituitary showed the scars of the previous necrotic regions. Sheehan and Murdoch (85) expressed the opinion that pregnancy is possible in any of these women in whom genital atrophy has not developed.

But what of the woman in whom genital atrophy has developed? Here we have the patient who has the most complete pituitary insufficiency that one can observe in human beings. Is it possible with end-organ replacement therapy to restore her uterus to such a normal degree that she could become pregnant? So far as we have been able to find, there has been only 1 reported instance of success in such a situation. Data on this patient have been reported by Sheehan and Murdoch (86).

The patient was a woman who at 24, 26 and 27 years of age had spontaneous deliveries following full-term pregnancies without any complications. At 29 years of age she had a twin pregnancy with spontaneous delivery at term. A few minutes after the birth of the children she became unconscious and did not recover until a long time after delivery of the placenta. Exact details of this collapse are lacking, but it was presumed to be due to obstetric shock or a severe postpartum hemorrhage. Signs and symptoms typical of severe anterior pituitary insufficiency then developed. There was complete absence of menstruation aside from one brief period of bleeding nine months after the delivery, which might have been related to trauma. She had typical menopausal flushing of the face for about two years and lost nearly all the hair from the axillae and mons pubis. Dyspareunia became gradually more severe. Marked sensitivity to cold and the characteristic changes of the skin developed.

At 32 years of age the patient first came under the observation of Sheehan and Murdoch. The vagina was shrunken and narrow. Its epithelium contained no glycogen and stained only yellow with iodine. The cervix was extremely small, and it was impossible to palpate the uterus or adnexa. The patient was very well studied, and treatment was then begun. Over a period of three and one-half months injections of an anterior pituitary extract were given, which produced slight leukorrhea, but the genitalia remained unchanged and the general condition of the patient was not affected. After this she was given injections of 50,000 international benzoate units of progynon B every two or three days for five weeks. This caused some increase of the leukorrhea and some lower abdominal pain. Five days after the injections had been stopped there was a slight vaginal bleeding lasting only a few hours, which cleared at the withdrawal of estrogenic hormone. Three weeks later the vagina and vulva did not show any remaining evidence of estrin stimulation, although for a month the uterus and cervix felt very slightly larger than before.

For the next four months the patient was left without treatment. It was decided to try to induce a pregnancy. The method proposed was first to produce and maintain with appropriate hormones a premenstrual condition of the uterus and endometrium, then to stimulate follicle production in the ovaries, and at this stage to provide opportunities for fertilization of any ova which might be produced. This treatment was begun four and one half years after the onset of superinvolution. Details can be found in Sheehan and Murdoch's (86) article. On the forty-first day after beginning treatment, she was examined. There had been marked improvement in the vulva and vagina, the cervix had the ordinary size and shape of a multiparous cervix, and the uterus was enlarged and slightly retroverted. On this occasion there was no pain with the examination, and the patient reported that the dyspareunia had disappeared. She had had an unexplained uterine hemorrhage on the thirty-first day of treatment. There were no subsequent hemorrhages or other symptoms, and about six weeks after finishing the course of injections, she became pregnant. About the middle of the pregnancy, the pubic and axillary hair was quite thick and the axillary perspiration had returned. She felt perfectly well and did not complain of cold or weakness. From about thirty-seven weeks onward there was a continuous slight flow of milk from both nipples. When the pregnancy was calculated to have reached thirty-nine weeks, labor was induced and she was delivered of a normal healthy baby. The puerperium was uneventful except that her breasts did not secrete the normal amount of milk. Following delivery there was a gradual return to her former state of pituitary insufficiency.

SUMMARY

We have made an effort to review the literature as it applies to the diagnosis and treatment of anterior pituitary insufficiency in the adult. We have tried to narrow the references to a point where anyone interested in reading further can find "key articles." We have listed the clinical and laboratory findings of pituitary insufficiency and have discussed the differential diagnosis between this condition and others with which it may be confused. Finally, we have touched on the subject of treatment, recording encouraging improvement within the last few years and expressing confidence in a more hopeful future.

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LETTER TO THE EDITOR

LACK OF EFFECT OF PARAHYDROXYPROPIOPHENONE IN HYPEROPHTHALMOPATHIC GRAVES' DISEASE AND DIABETES MELLITUS

To the Editor

Recent reports from France (1–4) concerning the ability of parahydroxypropiophenone (PHP) to inhibit the pituitary production of gonadotropin, thyrotropin, and somatotropin stimulated investigators in the United States to attempt verification of these assertions. Their reported results have almost universally failed to confirm the claims of the French workers (5–8). Our studies were undertaken with the intention of investigating, with clinical material, the action of this compound in patients with hyperophthalmopathic Graves' disease.

Thirteen patients with hyperopthalmopathic Graves' disease were treated exclusively with PHP, which was supplied by the Chilcott Laboratories. The duration of exophthalmos prior to treatment was from two months to twelve years. PHP was administered orally in doses ranging from 900 to 2,700 mg. per day. The medication was given over periods of from sixty to two hundred and twenty days. Seven of these patients (4 females, 3 males) showed exophthalmos with no thyrotoxicosis, whereas the other 6 (5 females, 1 male) were obviously thyrotoxic. Three of the thyrotoxic and 1 of the euthyroid patients had co-existent diabetes mellitus. In addition, PHP was administered to a thyrotoxic girl with no ophthalmopathy, and to a woman with the menopausal syndrome without Graves' disease.

The compound uniformly failed to produce a measurable decrease in exophthalmos, although the patients often stated that they believed their eyes felt much better, and in 1 instance there was some improvement in periorbital edema. In 11 patients the exophthalmos remained stationary, while in 2 there was progressive ophthalmopathy. A patient (white male, aged 40) whose exophthalmos began following thyroidectomy and who was euthyroid had Hertel exophthalmometer measurements of 25 mm. OD and 23 mm. OS at the time PHP was begun in a dose of 900 mg. per day. Six

weeks later the drug was increased to 1,800 mg. per day and after fourteen days on the increased dose, his eyes measured 28.5 and 24.5 mm. Another euthyroid patient (colored female, aged 30) had initial eye measurements of 28 and 29 mm. and after two months of PHP therapy at a dose level of 1,500 mg. per day, her ophthalmopathy had progressed to 29 and 30.5 mm. She was subsequently treated by irradiation of the pituitary and her condition improved.

There was no significant reduction in the toxicity of the hyperthyroid patients. One somewhat atypically thyrotoxic patient showed an increase in her BMR from +13 per cent to +32 per cent and an increase in 24-hour uptake of a tracer dose of 200 microcuries of I^{131} from 18 per cent to 34 per cent, while taking 900 mg. of PHP per day for four months. Another patient (colored female, aged 42) showed an increase in BMR from +47 per cent to +58 per cent, and in 24-hour I^{131} uptake from 43 per cent to 88 per cent, over a period of six months while receiving from 900 to 2,700 mg. per day.

Two of the patients with thyrotoxicosis and co-existing diabetes experienced no change in their insulin requirement. One patient (white male, aged 55), whose initial BMR was −9 per cent, had a slight reduction in protamine zinc insulin requirement from 30 to 24 units per day; whereas another (white female, aged 57) found it necessary to increase her daily insulin from 17 units to 20 units of protamine zinc insulin and 10 units of regular insulin while receiving 1,800 mg. of PHP per day.

The thyrotoxic patient without exophthalmos (white female, aged 21), who was treated with 1,200 mg. of PHP daily for sixty-eight days, showed no fall in BMR from +71 per cent, although she gained three pounds during this time. She later showed an excellent response to methylmercaptoimidazole (Methimazole, Lilly).

The patient (white female, aged 50) who received PHP for the menopausal syndrome in a dose of 900 mg. per day, experienced only questionable benefit for her symptoms of vasomotor instability. None of the female patients experienced any menstrual irregularities attributable to the medication.

In general, the compound was nontoxic. Transient nausea was noted in 2 patients when the drug was begun. One patient experienced mild diarrhea and headache while taking 1,800 mg. per day. In only 1 instance was a mild brief depression of polymorphonuclear leukocytes noted, but no depression of the total leukocyte count was observed.

It is apparent that in our experience PHP is essentially without beneficial effect upon hyperophthalmopathic Graves' disease. Schaffenburg *et al.* (7) and de Gennes *et al.* (9) each reported 2 cases, with similar results. There was no physiologic evidence of gonadotropin inhibition, insofar as there

were no significant changes in the menstrual history of the 8 women with normal cycles who received PHP.

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The Endocrine Society

34TH ANNUAL MEETING

JUNE 5-6-7, 1952

Headquarters: Palmer House, Chicago, Illinois.

Registration: Everyone attending the meetings is required to register. By action of the Council, non-members will be charged a registration fee of $5 for any part of the scientific sessions. Members should present their current membership cards when registering. Admission will be by badge only.

The Scientific Sessions: All scientific sessions will be held in the Red Lacquer Room. Sessions will begin promptly on schedule. Presentation of papers at all sessions will be LIMITED TO TEN MINUTES and authors are requested to adhere strictly to this schedule. Manuscripts of all papers should be submitted to the presiding officer or to the Secretary-Treasurer at the end of the presentation.

Annual Dinner: The Annual Dinner of the Society will be held on Friday evening, June 6, at 7:30 o'clock in the Red Lacquer Room, preceded by cocktails at 6:30 P.M. in the same room. Secure tickets at time of registration.

Local Arrangements: Dr. Willard O. Thompson, 700 North Michigan Avenue, Chicago, Illinois, is in charge of the local arrangements for the meetings.

Secretary-Treasurer: Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma.

(For the program, *see* the April issue of the Journal, page 463.)

1952 POSTGRADUATE ASSEMBLY

The Fifth Annual Postgraduate Assembly in Endocrinology and Metabolism will be held at the Roney Plaza Hotel, Miami Beach, Florida, November 3–8, 1952, immediately preceding the Southern Medical Association Meeting. See the Society journals for more detailed announcement or write the Secretary.

AMERICAN GERIATRICS SOCIETY

The ninth Annual Meeting of the American Geriatrics Society will be held in the La Salle Hotel, Chicago, Illinois, on June 5, 6 and 7, 1952, immediately preceding the American Medical Association sessions.

On Thursday, June 5, the Executive Committee will meet at 4:30 P.M. prior to the business meeting at 8:30 P.M.

Scientific sessions will open on Friday, June 6 and continue through Saturday, June 7, from 9 A.M. to 5 P.M. both days. Registration: Friday at 8 A.M.

An informal dinner will be held on Friday evening at 7:30, with a preceding gathering at 6:30 P.M.

All members of the society are urged to attend and non-members are invited to participate in the meeting. Hotel reservations should be made early by writing directly to Mr. M. P. Mathewson, La Salle Hotel, Chicago.

For further information, write the Secretary, Dr. Malford Thewlis, 25 Mechanic Street, Wakefield, R. I.

BOOKS RECEIVED

American Lecture Series. Charles C Thomas, Publisher, Springfield, Illinois.

Brain Tumors of Childhood. By HENRY M. CUNEO, M.D., Assistant Clinical Professor of Neurological Surgery, University of Southern California School of Medicine, and CARL W. RAND, M.D., Clinical Professor of Neurological Surgery, University of Southern California School of Medicine, Los Angeles. A monograph in American Lectures in Surgery, edited by Michael E. De Bakey, M.D., R. Glen Spurling, M.D., and B. Woodhall, M.D.; 236 pages; 80 illustrations; 1952; price $5.75.

Cellular Changes With Age. By WARREN ANDREW, Ph.D., M.D., Professor of Anatomy, The George Washington University School of Medicine, Washington, D. C. A monograph in American Lectures in Anatomy, edited by Otto F. Kampmeier, M.D.; 96 pages; 44 illustrations; 1952; price $2.75.

Diabetes and Pregnancy. By RALPH A. REIS, M.D., Professor of Obstetrics and Gynecology; EDWIN J. DECOSTA, M.D., Assistant Professor of Obstetrics and Gynecology; and M. DAVID ALLWEISS, M.D., Associate in Medicine, all of Northwestern University Medical School and Michael Reese Hospital, Chicago, Ill. A monograph in American Lectures in Gynecology and Obstetrics, edited by E. C. Hamblen, M.D.; 88 pages; 1952; price $2.50.

Etiology and Diagnosis in the Treatment of Infertility in Men. By ROBERT S. HOTCHKISS, M.D., Professor and Chairman, Department of Urology, New York University—Post Graduate Medical School, New York. A monograph in American Lectures in Endocrinology, edited by Willard O. Thompson, M.D.; 86 pages; 15 illustrations; 1952; price $2.50.

Neurosurgery: an Historical Sketch. By GILBERT HORRAX, M.D., Sc.D., Chief of Neurosurgical Service, The Lahey Clinic; Neurosurgeon, New England Baptist and New England Deaconess Hospitals, Boston, Mass. A monograph in American Lectures in Surgery, edited by Michael E. De Bakey, M.D., R. Glen Spurling, M.D., and B. Woodhall, M.D.; 142 pages; 69 illustrations; 181 references; 1952.

The Metabolic Response to Surgery. By FRANCIS D. MOORE, M.D., Moseley Professor of Surgery, Harvard Medical School; Surgeon-in-Chief, Peter Bent Brigham Hospital, Boston, Mass., and MARGARET R. BALL, A.B., Laboratory for Surgical Research, Peter Bent Brigham Hospital. A monograph in American Lectures in Surgery, edited by Michael E. De Bakey, M.D., R. Glen Spurling, M.D., and B. Woodhall, M.D.; 167 pages; 56 illustrations; 1952; price $7.50.

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